BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 June 2003 (12.06.2003)

PCT

(10) International Publication Number
WO 03/047547 A2

(51) International Patent Classification[7]: A61K 9/00

(21) International Application Number: PCT/EP02/13853

(22) International Filing Date: 6 December 2002 (06.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
01129034.3 6 December 2001 (06.12.2001) EP
02014624.7 2 July 2002 (02.07.2002) EP

(71) Applicants *(for all designated States except US)*: **SUNGENE GMBH & CO. KGaA** [DE/DE]; Correnssstr. 3, 06466 Gatersleben (DE). **GREENOVATION BIOTECH GMBH** [DE/DE]; Boetzinger Str. 29 B, 79111 Fribourg (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SALCHERT, Klaus-Dieter** [DE/DE]; Steinbergstr. 16, 06507 Gernrode (DE). **LEMKE, Rainer** [DE/DE]; Münzenberg 25, 06484 Quedlinburg (DE). **GEIGER, Michael** [DE/DE]; Siebennussbaumstr. 43/1, 74076 Heilbronn (DE). **TROPF, Susanne** [DE/DE]; Billungstr. 5, 06484 Quedlinburg (DE). **HERBERS, Karin** [DE/DE]; Am Hange 6, 06484 Quedlinburg (DE). **SCHLEDZ, Michael** [DE/DE]; Eisbrunnenweg 5, 79238 Ehrenkirchen (DE).

(74) Agent: **MECHNICH, Oliver**; c/o BASF Aktiengesellschaft, 67056 Ludwigshafen (DE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/047547 A2

(54) Title: NOVEL PRENYLTRANSFERASE

(57) Abstract: The present invention relates to new prenyltransferases, nucleic acids encoding the prenyltransferases, a method for producing vitamin E, plastoquinone and/or carotenoids by culturing geneticallymodified organisms, which have an increased prenyltransferase activity compared to the wild type, to the use of proteins as prenyltransferases and to the genetically modified organisms themselvesas well as their use in food and feed and as a source of natural vitamin E. The present invention further relates to the identification of plant prenyltransferases as a target for herbicides, to a method for identifying herbicidal agents based on plant prenyltransferases, and also to the use of compounds identified as herbicides via the abovementioned method.

Novel prenyltransferase

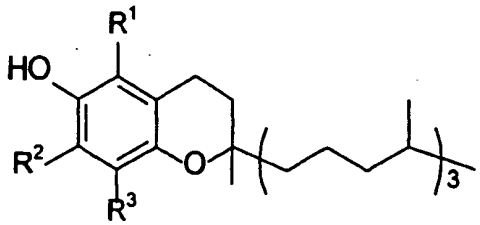
The present invention relates to new prenyltransferases, nucleic acids encoding the prenyltransferases, a method for producing vitamin E, plastoquinone and/or carotenoids by culturing genetically modified organisms, which have an increased prenyltransferase activity compared to the wild type, to the use of proteins as prenyltransferases and to the genetically modified organisms themselves as well as their use in food and feed and as a source of natural vitamin E. The present invention further relates to the identification of plant prenyltransferases as a target for herbicides, to a method for identifying herbicidal agents based on plant prenyltransferases, and also to the use of compounds identified as herbicides via the abovementioned method.

Due to their plastids, plants possess some biosynthetic pathways, which are, besides in cyanobacteria, unique in living organisms. Some plastidic compounds are indispensable for human and animal nutrition and are therefore called vitamins.Two essential lipophilic components for nutrition are provitamin A (beta-carotene) and vitamin E.

Vitamin E is predominantly delivered by the ingestion of vegetable oils. It plays an important role as a membrane-associated antioxidant scavenger. During past years several additional functions of vitamin E as anti-hypercholesterolemic and immunostimulatory agent in humans have been proposed (Beharka A, Redican S, Leka L and Meydani SN (1997). Vitamine E status and immune function. *Methods Enzymol.* 282, 247-263).

Vitamin E is classified by its pharmacological effect and chromanol ring structure and not by biosynthesis. It comprises a class of 8 lipid-soluble components, being subdivided into tocopherols and tocotrienols. While tocopherols share an isoprenoid side chain derived from phytyl-PP, tocotrienol side chains are derivates of geranylgeranyl-PP. The α, β, γ and δ-members of these subclasses differ in their degree of methylation in the 6-chromanol-ring structure.

The tocopherol group (1a-d) has a saturated side chain, and the tocotrienol group (2a-d) has an unsaturated side chain:



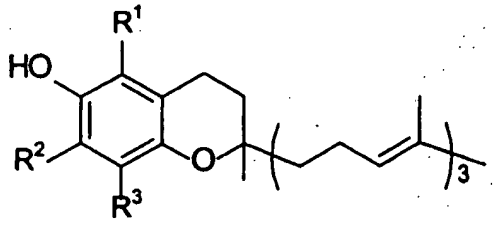
(1)

1a, α–tocopherol: $R^1 = R^2 = R^3 = CH_3$

1b, β–tocopherol: $R^1 = R^3 = CH_3$, $R^2 = H$

1c, γ–tocopherol: $R^1 = H$, $R^2 = R^3 = CH_3$

1d, δ–tocopherol: $R^1 = R^2 = H$, $R^3 = CH_3$



(2)

2a, α–tocotrienol: $R^1 = R^2 = R^3 = CH_3$

2b, β–tocotrienol: $R^1 = R^3 = CH_3$, $R^2 = H$

2c, γ–tocotrienol: $R^1 = H$, $R^2 = R^3 = CH_3$

2d, δ–tocotrienol: $R^1 = R^2 = H$, $R^3 = CH_3$

In the present invention, vitamin E means all of the aforementioned tocopherols and tocotrienols with vitamin E activity.

These compounds with vitamin E activity are important natural fat-soluble antioxidants. A vitamin E deficiency leads to pathophysiological situations in humans and animals. Vitamin E compounds therefore are of high economical value as additives in the food and feed sectors, in pharmaceutical formulations and in cosmetic applications.

Especially tocotrienols are discussed to have a higher potency in protection of eucaryotic cell membrane oxidation (Jandak J, Steiner M and Richardson PD (1989). Alpha-tocopherol, an effective inhibitor of platelet-adhesion. *Blood* 73, 141-149; Kamat JP, Sarma HD, Devasagayam TPA, Nesaretnam K and Basiron Y (1997). Tocotrienols from palm oil as effective inhibitors of protein oxidation and lipid peroxidation in rat liver microsomes. *Mol. Cell Biochem.* 170, 131-138). Moreover, tocotrienols have been shown to suppress cholesterol biosynthesis by post-transcriptional inhibition of hydroxymethyl glutaryl-CoA-reductase. They are involved in the degradation of artherogenic apolipoproteine B and the reduction of lipoprotein plasma levels (Parker RA, Pearce BC, Clark RW, Gordon DA and Wright JJK (1993). Tocotrienols regulate cholesterol production in mammalian cells by post-transcriptional suppression of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* 268, 11230-11238; Wang Q, Theriault A, Gapor A and Adeli K. (1998). Effects of tocotrienol on the intracellular translocation and degradation of apolipoprotein B: possible involvement of a proteasome independent pathway. *Biochem. Biophys.*

Res. Com. 246, 640-643), whereas tocopherols don't have these pharmacological effects (Qureshi AA, Bradlow BA, Brace L, Manganello J, Peterson DM, Pearce BC Wright JJ, Gapor A and Elson CE (1995). Response of hypercholesterolemic subjects to administration of tocotrienols. *Lipids* 30, 1171-1177).

In addition, tocotrienols have been suggested to have an anti-thrombotic and anti-tumor effect indicating that they may serve as an effective agent in the prevention or treatment of cardiovascular disease and cancer (Theriault A, Chao JT, Wang Q, Gapor A and Adeli K (1999). Tocotrienol: a review of its therapeutic potential. *Clin. Biochem.* 32, 309-319).

In plastids of plants many isoprenoid pathways are localized, which are interconnected by their substrates, end products and by regulation. These are, e.g. monoterpene-, diterpene-, giberillic acid-, abscisic acid-, chlorophyll-, phylloquinone-, carotenoid-, tocopherol-, tocotrienol- and plastoquinone-biosynthesis. In all these pathways prenyltransferases are involved in the biosynthesis of these compounds. With respect to the length of their side chains diterpenes, chlorophylls, phylloquinones, tocopherols and tocotrienols can be arranged into a C_{20}-group of isoprenoids. Another classification by degree of desaturation of the side chain, would arrange e.g. chlorophylls, phylloquinones and tocopherols into a phytyl-group and e.g. diterpenes, tocotrienols, plastoquinones and carotenoids into a group of desaturated isoprenoid compounds.

Classification by head groups would arrange tocopherols, tocotrienols and plastoquinones (Fig. 1) in one group, being quinones with antioxidant properties and having homogentisic acid as a precursor. Plastoquinones are important components of the quinone-pool in the photosynthetic electron transport chains of plastids, also interfering in the biosynthesis of provitamin A (beta-carotene; Norris SR, Barrette TR and DellaPenna D. (1995). Genetic dissection of carotenoid synthesis in *Arabidopsis* defines plastoquinone as an essential component of phytoene desaturation. *Plant Cell* 7, 2139-2149).

prenyltransferase s involved in the formation of tocopherols and tocotrienols/plastoquinones are expected to mark a branching point of these pathways (Fig.1). These enzymes are supposed to catalyze the condensation of saturated/desaturated isoprenoid-diphosphates with homogentisic acid by a complex prenylation/phytylation-reaction involving decarboxylation of the aromatic moiety.

An economical method for producing vitamin E, plastoquinone and/or carotenoids compounds and food- and feedstuffs with increased vitamin E content are therefore very important.

Particularly economical methods are biotechnological methods utilizing vitamin E-producing

organisms which are either natural or optimized by genetic modification.

A *pds2*-mutant has been described being deficient in plastoquinones and impaired in the carotenoid level (Norris *et al.*, 1995). This mutant was found in a screening assay of Arabidopsis mutants that disrupt the plastoquinone-dependent desaturation of carotenoids and lead to the accumulation of phytoene, the first carotenoid in this pathway. The undetectable amounts of tocopherols in the mutant, arose the question of a multifunctional phytyl/prenyl transferase for plastoquinone, tocotrienol and tocopherol biosynthesis.

There is a constant need for providing novel enzyme activities and thus alternative methods with advantageous properties for producing vitamin E, plastoquinone and/or carotenoids in transgenic organisms.

Attempts are known to achieve an increase in the flow of metabolites so as to increase the tocopherol and/or tocotrienol content by overexpressing Phytyl/prenyltransferasegenes in transgenic organisms.

WO 00/63391, WO 00/68393, WO 01/62781 and WO 02/33060 disclose the cloning and overexpression in organisms of genes encoding phytyl/prenyltransferases from Synechocystis and Arabidopsis thaliana. It is postulated that the phytyltransferases which catalyse the conversion of homogentisate and phytyl pyrophosphate into 2–methyl-6-phytyl-hydroquinone catalyse also the conversion of homogentisate and geranylgeranyl pyrophosphate into 2–methyl-6-geranylgeranyl-hydroquinone.

Schledz M, Seidler A, Beyer P and Neuhaus G ((2001), *FEBS Letters* 499, 15-20) identified a tocopherol phytyltransferase from Synechocystis sp.PCC 6803. Analyzing the deletion mutant of *slr1736* by its loss of function, it was impaired in tocopherol content. However plastoquinone and carotenoid levels remained unchanged.

Performing a FASTA-alignment of SLR1736 peptide sequence against the genomic database of Arabidopsis thaliana highest homology (51,22 % identity in a 41 amino acids overlap) lightens up to a hypothetical protein, coded by BAC-clone F19F24.15 and located on chromosome 2. With some corrections to the exon/intron-structure, cloning of the cDNA and recombinant expression of the protein it could be currently demonstrated by *in vitro*-enzymatic assays with radioactive precursors that this protein is the tocopherol phytyltransferase of *Arabidopsis*. In these assays specificity for GGPP or solanesyl-PP of Arabidopsis tocopherol phytyltransferase was not detectable (Collakova E and DellaPenna D (2001). Isolation and functional analysis of homogentisate phytyltransferase from *Synechocystis* sp. PCC 6803 and Arabidopsis. *Plant Physiol.* 127,

1113-1124). The authors assumed that a different prenyltransferase has to exist that catalyzes the prenylation reaction of plastoquinone biosynthesis and that the tocopherol phytyltransferase catalyzes the formation of tocopherols. Tocotrienols are not mentioned in this context.

The known phytyl/prenyltransferases of the prior art show the disadvantage of higher selectivities for saturated prenyl substrates such as phytyl pyrophosphate and lower selectivities for the unsaturated prenyl substrates such as geranylgeranyl pyrophosphate or solanesyl pyrophosphate leading to an higher increase of tocopherols compared to tocotrienols.

It is an object of the present invention to provide prenyltransferases with higher selectivities for unsaturated prenyl substrates such as geranylgeranyl pyrophosphate or solanesyl pyrophosphate and lower selectivities for the saturated prenyl substrates such as phytyl pyrophosphate.

We have found that this object is achieved by proteins with prenyltransferase activity comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 50 % identity at amino acid level with sequence SEQ. ID. NO. 2.

prenyltransferase activity means the enzyme activity of a prenyltransferase.

A prenyltransferase means a protein which has the enzymic activity of converting homogentisate and geranylgeranyl pyrophosphate into 2–methyl-6-geranylgeranyl-hydroquinone.

Accordingly, prenyltransferase activity means the amount of homogentisate or geranylgeranyl pyrophosphate converted or the amount of 2–methyl-6-geranylgeranyl-hydroquinone formed by the protein prenyltransferase in a particular time.

The prenyltransferases according to the invention are capable of converting homogentisate and geranylgeranyl pyrophosphate into 2-methyl-6-geranylgeranylhydroquinone and/or of converting homogentisate and solanesyl pyrophosphate into 2-methyl-6-solanesyl-hydroquinone.

The prenyltransferases according to the invention are, even with a less selectivity, also capable of converting homogentisate and phytyl pyrophosphate into 2-methyl-6-phytyl-hydroquinone. In organisms with low content of geranylgeranyl pyrophosphate and/or solanesyl pyrophosphate as substrates, such as rape, also phytyl pyrophosphate will be converted.

SEQ ID NO: 2 represents the amino acid sequence of the prenyltransferase of Arabidopsis thaliana, SEQ ID NO: 1 the corresponding encoding nucleic acid sequence as one example of

the prenyltransferases and corresponding prenyltransferase genes according to the invention. .

Surprisingly, it was found that the sequence SEQ. ID. NO. 1 could be established from BAC clone F26K24 (accession no. AC016795), not being recognized as part of a coding sequence and the Arabidopsis genomic DNA, P1 clone MEC18 (acession no. AP002040). The predicted coding sequence of MEC18.5 was only partly correct as the exon/intron borders were set falsely.

The sequence SEQ. ID. No. 2 of the prenyltransferase from *Arabidopsis thaliana* has a low identity of 44 % at the amino acid level with the amino acid sequence of the phytyltransferase from *Arabidopsis thaliana* which is known from the prior art.

The prenyltransferases according to the invention comprise the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 50 %, preferably at least 60%, more preferably at least 70%, more preferably at least 80%, still more preferably at least 90%, most preferably at least 95%, identity at amino acid level with sequence SEQ. ID. NO. 2.

The proteins which comprise a sequence derived from SEQ. ID. NO. 2, which is at least 50%, preferably at least 60%, more preferably at least 70%, more preferably at least 80%, still more preferably at least 90%, most preferably at least 95%, identical at the amino acid level to the sequence SEQ. ID. NO. 2 and which also have the enzymic property of a prenyltransferase may also be prepared, as mentioned above, by artificial variations starting from the SEQ. ID. NO. 2, for example by substitution, insertion or deletion of amino acids.

The term "substitution" means in the description the replacement of one or more amino acids by one or more amino acids. Preference is given to carrying out "conservative" replacements in which the amino acids replaced has a property similar to that of the original amino acid, for example replacement of Glu by Asp, Gln by Asn, Val by Ile, Leu by Ile, Ser by Thr.

Deletion is the replacement of an amino acid by a direct bond. Preferred positions for deletions are the polypeptide termini and the junctions between the individual protein domains.

Insertions are insertions of amino acids into the polypeptide chain, with a direct bond formally being replaced by one or more amino acids.

Identity between two proteins means the identity of the amino acids over the in each case entire length of the protein, in particular the identity which is calculated by comparison with the aid of the Vector NTI Suite 7.1 Software of the company Informax (USA) using the Clustal method

(Higgins DG, Sharp PM. Fast and sensitive multiple sequence alignments on a microcomputer. Comput Appl. Biosci. 1989 Apr;5(2):151-1) with the parameters set as follows:

Multiple alignment parameter:	
Gap opening penalty	10
Gap extension penalty	10
Gap separation penalty range	10
Gap separation penalty	off
% identity for alignment delay	50
Residue specific gaps	off
Hydrophilic residue gap	off
Transition weighing	0

Pairwise alignment parameter:	
FAST algorithm on	
K-tuple size	1
Gap penalty	3
Window size	5
Number of best diagonals	5

Accordingly, a protein which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2 means a protein which, when comparing its sequence with the sequence SEQ. ID. NO. 2, is at least 50% identical, in particular according to the above program algorithm using the above set of parameters.

Further natural examples of prenyltransferases and prenyltransferase genes according to the invention can readily be found, for example, in various organisms, in particular in plants, whose genomic sequence is known by comparing the identity of the amino acid sequences or of the corresponding backtranslated nucleic acid sequences from databases with the sequence of SEQ. ID. NO. 2, in particular according to the above program algorithm using the above set of parameters.

Further natural examples of prenyltransferases and the corresponding prenyltransferase genes according to the invention are, for example, sequences from

Brassica (EST sequences:SEQ ID NO: 7 to SEQ ID NO: 14), or

Soya (EST sequence:SEQ ID NO: 15)

Further natural examples of prenyltransferases and prenyltransferase genes can furthermore readily be found in various organisms, in particular plants, whose genomic sequence is unknown by hybridization techniques in a manner known per se, for example starting from the sequence SEQ. ID. No. 1.

The hybridization may be carried out under moderate (low stringency) or, preferably, under stringent (high stringency) conditions.

Such hybridization conditions are described, inter alia, in Sambrook, J., Fritsch, E.F., Maniatis, T., in: Molecular Cloning (A Laboratory Manual), 2nd edition, Cold Spring Harbor Laboratory Press, 1989, pages 9.31-9.57 or in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6.

By way of example, the conditions during the washing step may be selected from the range of conditions which is limited by those with low stringency (with 2X SSC at 50_C) and those with high stringency (with 0.2X SSC at 50_C, preferably at 65_C) (20X SSC: 0.3 M sodium citrate, 3 M sodium chloride, pH 7.0).

In addition, the temperature may be raised during the washing step from moderate conditions at room temperature, 22_C, to stringent conditions at 65_C.

Both parameters, salt concentration and temperature, may be varied simultaneously and it is also possible to keep one of the two parameters constant and to vary only the other one. It is also possible to use denaturing agents such as, for example, formamide or SDS during hybridization. In the presence of 50% formamide, the hybridization is preferably carried out at 42_C.

Some exemplary conditions for hybridization and washing step are listed below:

(1) hybridization conditions with, for example

(i) 4X SSC at 65_C, or

(ii) 6X SSC at 45_C, or

(iii) 6X SSC at 68_C, 100 mg/ml denatured fish sperm DNA, or

(iv) 6X SSC, 0.5% SDS, 100 mg/ml denatured fragmented salmon sperm DNA at 68_C, or

(v) 6X SSC, 0.5% SDS, 100 mg/ml denatured fragmented salmon sperm DNA, 50% formamide at 42_C, or

(vi) 50% formamide, 4X SSC at 42_C, or

(vii) 50% (vol/vol) formamide, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer pH 6.5, 750 mM NaCl, 75 mM sodium citrate at 42_C, or

(viii) 2X or 4X SSC at 50_C (moderate conditions), or

(ix) 30 to 40% formamide, 2X or 4X SSC at 42_C (moderate conditions).

(2) Washing steps of 10 minutes each with, for example

(i) 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50_C, or

(ii) 0.1X SSC at 65_C, or

(iii) 0.1X SSC, 0.5% SDS at 68_C, or

(iv) 0.1X SSC, 0.5% SDS, 50% formamide at 42_C, or

(v) 0.2X SSC, 0.1% SDS at 42_C, or

(vi) 2X SSC at 65_C (moderate conditions).

Preferred proteins with prenyltransferase activity are proteins from plants, cyanobacteria, mosses or algae, particular preferred from plants. A particular preferred protein comprises the amino acid sequence SEQ ID NO: 2.

The invention further relates to nucleic acids encoding a prenyltransferase according to the invention. All of the nucleic acids mentioned in the description may be, for example, an RNA sequence, DNA sequence or cDNA sequence.

Suitable nucleic acid sequences can be obtained, for example, by backtranslating the polypeptide sequence according to genetic code. For this, preference is given to using those codons

which are used frequently according to the organism-specific codon usage. The codon usage can be readily determined on the basis of computer analyses of other known genes of the organisms in question.

If, for example, the protein is to be expressed in a plant, it is frequently advantageous to use the codon usage of said plant for backtranslation.

In a preferred embodiment, the nucleic acids encode a prenyltransferase from plants, cyanobacteria, mosses or algae. In a particularly preferred embodiment, the nucleic acids encode prenyltransferases of plants, such as the nucleic acids which can be easily found in Brasica plants, using the ESTs of SEQ ID NO: 7 to SEQ ID NO: 14 as a gene probe or the nucleic acids which can be easily found in soya plants, using the EST of SEQ ID NO: 15 as a gene probe.

In a particularly preferred embodiment, the nucleic acid comprises the sequence SEQ. ID. NO. 1. Particular preferred, the nucleic acid consists of the sequence SEQ. ID. NO. 1.The sequence SEQ. ID. NO. 1 is the cDNA sequence from *Arabidopsis thaliana* which encodes the prenyltransferase of the sequence SEQ. ID. NO. 2.

All of the abovementioned prenyltransferase genes can furthermore be prepared in a manner known per se from the nucleotide building blocks by chemical synthesis, for example by fragment condensation of individual overlapping complementary nucleic acid building blocks of the double helix. The chemical synthesis of oligonucleotides may be carried out, for example, in a known manner according to the phosphoramidite method (Voet, Voet, 2nd edition, Wiley Press New York, pages 896-897). The annealing of synthetic oligonucleotides and filling-in of gaps with the aid of the Klenow fragment of DNA polymerase and ligation reactions and also general cloning methods are described in Sambrook et al. (1989), Molecular cloning: A laboratory manual, Cold Spring Harbor Laboratory Press.

The invention further relates to a method for producing vitamin E, plastoquinone and/or carotenoids by culturing organisms which have, compared to the wild type, an increased prenyltransferase activity, said prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

Increased prenyltransferase activity compared to the wild type means that the amount of homogentisate or geranylgeranyl pyrophosphate converted or the amount of 2–methyl-6-geranylgeranyl-hydroquinone formed is increased by the protein prenyltransferase in a particular

time, in comparison with the wild type.

This increase in prenyltransferase activity is preferably at least 5%, further preferably at least 20%, further preferably at least 50%, further preferably at least 100%, more preferably at least 300%, still more preferably at least 500%, in particular at least 600%, of the prenyltransferase activity of the wild type.

A "wild type" means the corresponding genetically unmodified starting organism. Preferably and, in particular, in cases in which the organism or the wild type cannot be classified unambiguously, wild type, in the case of increasing the prenyltransferase activity and of increasing the vitamin E, plastoquinone and/or carotenoid content, means a reference organism. This reference organism is preferably *Brassica napus cv Westar*.

Depending on the context, the term "organism" means the wild type/starting organism or the genetically modified organism or both.

In the event the starting organism provides as wild type no prenyltransferase activity, the term "increasing the prenyltransferase activity" means also "causing the prenyltransferase activity".

The prenyltransferase activity may be increased in various ways, for example by eliminating inhibiting regulatory mechanisms at the translation and protein levels or by increasing the gene expression of a nucleic acid encoding a prenyltransferase compared to the wild type, for example by inducing the prenyltransferase gene via activators or by introducing into the organism nucleic acids encoding a prenyltransferase.

According to the invention, increasing the gene expression of a nucleic acid encoding a prenyltransferase also means manipulating the expression of the endogenous prenyltransferases intrinsic to the organism, in particular to plants. This may be achieved, for example, by modifying the promoter DNA sequence of genes encoding prenyltransferases. Such a modification which leads to a modified or preferably increased rate of expression of at least one endogenous prenyltransferase gene may be carried out by deleting or inserting DNA sequences.

As described above, it is possible to modify expression of at least one endogenous prenyltransferase by applying exogenous stimuli. This may be carried out by particular physiological conditions, i.e. by applying foreign substances.

Furthermore, it is possible to achieve a modified or increased expression of at least one endogenous prenyltransferase gene by the interaction of a regulatory protein which is modified or is

not present in the untransformed organism with the promoter of said genes.

Such a regulatory protein may be a chimeric protein comprising a DNA-binding domain and a transcriptional activator domain, as described, for example, in WO 96/06166.

In a preferred embodiment, the prenyltransferase activity is increased compared to the wild type by increasing the gene expression of a nucleic acid encoding a prenyltransferase, said prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

In a further preferred embodiment, gene expression of a nucleic acid encoding a prenyltransferase is increased by introducing into the organism nucleic acids encoding prenyltransferases, said prenyltransferases comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids which are at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

For this purpose, it is in principle possible to use any prenyltransferase gene of the invention, i.e. any nucleic acids encoding a prenyltransferase, said prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

In the case of genomic prenyltransferase nucleic acid sequences from eukaryotic sources, which contain introns, preferably already processed nucleic acid sequences such as the corresponding cDNAs are to be used, if the host organism is unable to or cannot be enabled to express the corresponding prenyltransferase.

In this preferred embodiment, the transgenic organisms of the invention thus contain, compared to the wild type, at least one further prenyltransferase gene. In this preferred embodiment, the genetically modified organism of the invention has accordingly at least one transgenic or exogenous nucleic acid encoding a prenyltransferase or at least two endogenous nucleic acids encoding a prenyltransferase.

Suitable and preferred nucleic acids are described above. In a particularly preferred embodiment, a nucleic acid comprising the sequence SEQ. ID. NO. 1 is introduced into the organism.

According to the invention, organisms means preferably prokaryotic organisms or eukaryotic organisms, such as, for example, bacteria, yeasts, algae, mosses, fungi or plants, which are capable of producing vitamin E, either as wild type or enabled by genetic modification. Preferred organisms are photosynthetically active organisms such as, for example, cyanobacteria, mosses, algae or plants which, even as a wild type, are capable of producing vitamin E.

Particularly preferred organisms are plants.

Preferred plants are tagetes, sunflower, Arabidopsis, tobacco, red pepper, soybean, tomato, egg-plant, paprika, carrot, potato, corn, lettuce and cabbage species, cereals, alfalfa, oats, barley, rye, wheat, triticale, millet, rice, lucerne, flax, cotton, hemp, rape, canola, sugar beet, sugar cane, nut and vine species or wood plants, such as, for example, aspen or yew.

Particular preference is given to *Arabidopsis thaliana*, *Tagetes erecta*, *Brassica napus*, *Nicotiana tabacum*, sunflower, canola, potato and soybean.

As mentioned above, a wild type means the corresponding genetically unmodified starting organisms. Preferably and, in particular, in cases in which the organism or the wild type cannot be classified unambiguously, wild type means, as mentioned above, in the case of increasing the prenyltransferase activity and of increasing the vitamin E content, a reference organism. This reference organism is preferably *Brassica napus cv Westar.*

The prenyltransferase activity in the organism of the invention and in the reference organism is determined preferably under the following conditions:

The activity of prenyltransferase in the particular organism is measured after isolating the chloroplasts. For this purpose, the leaf material is homogenized in a Waring blender with 5-10 times the amount (e.g. 5 g in 50 ml) of isolation buffer (50 mM Tris-HCl pH 8.0, 600 mM sorbitol, 0.1% ascorbate, 0.05% mercaptoethanol, 1 mM aminocaproic acid). The homogenate is filtered through 4 layers of Miracloth or nylon (50 mm). The filtrate is centrifuged at 4_C and 6000xg for 10 minutes. The supernatant is discarded. The pellet is washed in 5 ml of 0.1 M potassium phosphate buffer pH 8.0 and a protease inhibitor mixture (1 tablet/50 ml), the chloroplasts being resuspended using a brush. Finally, the pellet is resuspended in 0.6 - 1 ml of the potassium phosphate buffer pH 8.0 with protease inhibitors by using said brush. The sample is transferred to 2 ml Eppendorf reaction vessels and admixed with 0.2% CHAPS. The sample is agitated at 4_C for 30 – 60 minutes (Vortex Genie2 level 1). The lysate is centrifuged at 4_C and 13 000 rpm for 10 minutes. The supernatant is removed and its protein concentration is deter-

mined according to standard methods.

For the enzyme assay, 145 ml of this protein suspension are admixed with:

200 ml 125 mM tricine-NaOH pH 8.0
100 ml 1.25 M sorbitol
10 ml 50 mM $MgCl_2$
20 ml 250 mM ascorbic acid (always freshly prepared)
10 ml 5 mM substrate C^{14}-labeled geranylgeranyl pyrophosphate or solanesyl pyrophosphate or phytyl pyrophopsphate or homgentisate.

The mixture is incubated at 30_C for 3 - 48 hours.

The enzyme assay is stopped by adding 750 ml of chloroform/methanol (1:2) and 750 ml of 0.9% NaCl. The phases are separated by centrifugation at 13 000 rpm for 2 minutes. The lower chloroform phase is transferred to a new Eppendorf reaction vessel and evaporated to dryness in a Speed Vac. The residue is taken up in 20 ml of ether and analyzed via thin layer chromatography (solid phase: HPTLC plates: silica gel 60 F_{254}; liquid phase: toluene). The controls applied are 2-methyl-6-geranylgeranylhydroquinone, 2-methyl-6-solanesylhydroquinone or 2-methyl-6-phytylhydroquinone respectively.

After the run, the thin layer plate is dried and then subjected to autoradiography.

In the method for producing vitamin E, plastoquinone and/or carotenoids according to the invention, the cultivation step of the genetically modified organisms, also referred to as transgenic organisms hereinbelow, is preferably followed by harvesting said organisms and isolating vitamin E, plastoquinone and/or carotenoids from said organisms.

The organisms are harvested in a manner known per se and appropriate for the particular organism. Microorganisms such as bacteria, mosses, yeasts and fungi or plant cells which are cultured in liquid media by fermentation may be removed, for example, by centrifugation, decanting or filtration. Plants are grown on solid media in a manner known per se and harvested accordingly.

Vitamin E, plastoquinone and/or carotenoids are isolated from the harvested biomass in a manner known per se, for example by extraction and, where appropriate, further chemical or physical purification processes such as, for example, precipitation methods, crystallography, thermal separation methods such as rectification methods or physical separation methods such as, for

example, chromatography.

Vitamin E is isolated from oil-containing plants, for example, preferably by chemical conversion and distillation from vegetable oils or from the steam distillates obtained in the deodorization of vegetable oils (deodorizer condensates).

Further methods of isolating vitamin E from deodorizer condensates are described, for example, in DE 31 26 110 A1, EP 171 009 A2, GB 2 145 079, EP 333 472 A2 and WO 94/05650.

The present invention furthermore relates to the use of proteins comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2 and having the enzymic activity of a prenyltransferase as prenyltransferase.

The prenyltransferases according to the invention are capable of and may be used for converting homogentisate and geranylgeranyl pyrophosphate into 2-methyl-6-geranylgeranylhydroquinone and/or of converting homogentisate and solanesyl pyrophosphate into 2-methyl-6-solanesyl-hydroquinone.

The prenyltransferases according to the invention are, even with a less selectivity, also capable of and may be used for converting homogentisate and phytyl pyrophosphate into 2-methyl-6-phytyl-hydroquinone. In organisms with low content of geranylgeranyl pyrophosphate and/or solanesyl pyrophosphate as substrates, such as rape, also phytyl pyrophosphate will be converted.

The present invention further relates to the use of nucleic acids encoding the abovementioned proteins for expressing proteins having a prenyltransferase activity.

The transgenic organisms, in particular plants, are preferably prepared by transforming the starting organisms, in particular plants, with a nucleic acid construct containing the above-described, prenyltransferase encoding nucleic acids which are functionally linked to one or more regulatory signals ensuring transcription and translation in organisms.

These nucleic acid constructs in which the coding nucleic acid sequence is functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, in particular in plants, are also referred to as expression cassettes hereinbelow.

Accordingly, the invention further relates to nucleic acid constructs, in particular to nucleic acid constructs functioning as expression cassette, which comprise a prenyltransferase encoding nucleic acid which is functionally linked to one or more regulatory signals ensuring transcription and translation in organisms, in particular in plants.

The regulatory signals preferably comprise one or more promoters ensuring transcription and translation in organisms, in particular in plants.

The expression cassettes include regulatory signals, i.e. regulatory nucleic acid sequences, which control expression of the coding sequence in the host cell. According to a preferred embodiment, an expression cassette comprises upstream, i.e. at the 5' end of the coding sequence, a promoter and downstream, i.e. at the 3' end, a polyadenylation signal and, where appropriate, further regulatory elements which are operatively linked to the coding sequence for at least one of the above-described genes located in between. Operative linkage means the sequential arrangement of promoter, coding sequence, terminator and, where appropriate, further regulatory elements in such a way that each of the regulatory elements can properly carry out its function in the expression of the coding sequence.

When the organism used is plants, the nucleic acid constructs and expression cassettes of the invention preferably contain a nucleic acid encoding a plastid transit peptide ensuring localization in plastids.

The preferred nucleic acid constructs, expression cassettes and vectors for plants and methods for preparing transgenic plants and also the transgenic plants themselves are described by way of example below.

The sequences preferred for operative linkage, but not limited thereto, are targeting sequences for ensuring subcellular localization in the apoplasts, in the vacuole, in plastids, in the mitochondrion, in the endoplasmic reticulum (ER), in the nucleus, in elaioplasts or in other compartments and translation enhancers such as the tobacco mosaic virus 5'-leader sequence (Gallie et al., Nucl. Acids Res. 15 (1987), 8693-8711).

A suitable promoter of the expression cassette is in principle any promoter which is able to control the expression of foreign genes in plants.

"Constitutive" promoter means those promoters which ensure expression in numerous, preferably all, tissues over a relatively long period of plant development, preferably during the entire

plant development.

Preference is given to using, in particular, a promoter from plants or a promoter originating from a plant virus. Preference is in particular given to the promoter of the 35S transcript of the CaMV cauliflower mosaic virus (Franck et al. (1980) Cell 21:285-294; Odell et al. (1985) Nature 313:810-812; Shewmaker et al. (1985) Virology 140:281-288; Gardner et al. (1986) Plant Mol Biol 6:221-228) or the 19S CaMV promoter (US 5,352,605; WO 84/02913; Benfey et al. (1989) EMBO J 8:2195-2202).

Another suitable constitutive promoter is the Rubisco small subunit (SSU) promoter (US 4,962,028), the leguminB promoter (GenBank Acc. No. X03677), the Agrobacterium nopaline synthase promoter, the TR double promoter, the agrobacterium OCS (octopine synthase) promoter, the ubiquitin promoter (Holtorf S et al. (1995) Plant Mol Biol 29:637-649), the ubiquitin 1 promoter (Christensen et al. (1992) Plant Mol Biol 18:675-689; Bruce et al. (1989) Proc Natl Acad Sci USA 86:9692-9696), the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US 5,683,439), the promoters of the vacuolar ATPase subunits or the promoter of a proline-rich wheat protein (WO 91/13991), the Pnit promoter (Y07648.L, Hillebrand et al. (1998), Plant. Mol. Biol. 36, 89-99, Hillebrand et al. (1996), Gene, 170, 197-200) and other promoters of genes whose constitutive expression in plants is known to the skilled worker.

The expression cassettes may also contain a chemically inducible promoter (review: Gatz et al. (1997) Annu Rev Plant Physiol Plant Mol Biol 48:89-108) which may be used to control expression of the prenyltransferase gene in the plants at a particular time. Promoters of this kind, such as, for example, the PRP1 promoter (Ward et al. (1993) Plant Mol Biol 22:361-366), salicylic acid-inducible promoter (WO 95/19443), a benzenesulfonamide-inducible promoter (EP 0 388 186), a tetracycline-inducible promoter (Gatz et al. (1992) Plant J 2:397-404), an abscisic acid-inducible promoter (EP 0 335 528) and an ethanol- or cyclohexanone-inducible promoter (WO 93/21334), may likewise be used.

Further preference is given to promoters which are induced by biotic or abiotic stress, such as, for example, the pathogen-inducible promoter of the PRP1 gene (Ward et al. (1993) Plant Mol Biol 22:361-366), the heat-inducible tomato hsp70 or hsp80 promoter (US 5,187,267), the cold-inducible potato alpha-amylase promoter (WO 96/12814), the light-inducible PPDK promoter or the injury-induced pinII promoter (EP 375091).

Pathogen-inducible promoters comprise those of genes which are induced due to a pathogen attack, such as, for example, genes of PR proteins, SAR proteins, b-1,3-glucanase, chitinase, etc. (for example, Redolfi et al. (1983) Neth J Plant Pathol 89:245-254; Uknes, et al. (1992) The

Plant Cell 4:645-656; Van Loon (1985) Plant Mol Viral 4:111-116; Marineau et al. (1987) Plant Mol Biol 9:335-342; Matton et al. (1987) Molecular Plant-Microbe Interactions 2:325-342; Somssich et al. (1986) Proc Natl Acad Sci USA 83:2427-2430; Somssich et al. (1988) Mol Gen Genetics 2:93-98; Chen et al. (1996) Plant J 10:955-966; Zhang and Sing (1994) Proc Natl Acad Sci USA 91:2507-2511; Warner, et al. (1993) Plant J 3:191-201; Siebertz et al. (1989) Plant Cell 1:961-968 (1989).

Likewise included are injury-inducible promoters such as those of the pinII gene (Ryan (1990) Ann Rev Phytopath 28:425-449; Duan et al. (1996) Nat Biotech 14:494-498), the wun1 and wun2 genes (US 5,428,148), the win1 and win2 genes (Stanford et al. (1989) Mol Gen Genet 215:200-208), the systemin gene (McGurl et al. (1992) Science 225:1570-1573), the WIP1 gene (Rohmeier et al. (1993) Plant Mol Biol 22:783-792; Eckelkamp et al. (1993) FEBS Letters 323:73-76), the MPI gene (Corderok et al. (1994) The Plant J 6(2):141-150), and the like.

Further examples of suitable promoters are fruit ripening-specific promoters such as, for example, the fruit ripening-specific promoter from tomato (WO 94/21794, EP 409 625). Development-dependent promoters partly include the tissue-specific promoters, since individual tissues are naturally formed in a development-dependent manner.

Furthermore, preference is given in particular to those promoters which ensure expression in tissues or parts of the plant, in which, for example, biosynthesis of vitamin E or of the precursors thereof takes place. Preference is given, for example, to promoters with specificities for the anthers, ovaries, flowers, leaves, stems, roots and seeds.

Seed-specific promoters are, for example, the phaseoline promoter (US 5,504,200; Bustos MM et al. (1989) Plant Cell 1(9):839-53), the promoter of the 2S albumin gene (Joseffson LG et al. (1987) J Biol Chem 262:12196-12201), the legumin promoter (Shirsat A et al. (1989) Mol Gen Genet 215(2): 326-331), the USP (unknown seed protein) promoter (Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-67), the promoter of the napin gene (US 5,608,152; Stalberg K et al. (1996) L Planta 199:515-519), the sucrose-binding protein promoter (WO 00/26388) and the legumin B4 promoter (LeB4; Bäumlein H et al. (1991) Mol Gen Genet 225: 121-128; Baeumlein et al. (1992) Plant Journal 2(2):233-9; Fiedler U et al. (1995) Biotechnology (NY) 13(10):1090f), the Arabidopsis oleosin promoter (WO 98/45461), the Brassica Bce4 promoter (WO 91/13980) and the vicillin promoter (Weschke et al. 1988, Biochem. Physiol. Pflanzen 183, 233-242; Bäumlein H et al. (1991) Mol Gen Genet 225(3):459-67).

Further suitable seed-specific promoters are those of the genes coding for high molecular weight glutenine (HMWG), gliadin, branching enzyme, ADP glucose pyrophosphatase (AGPase)

and starch synthase. Preference is further given to promoters which allow seed-specific expression in monocotyledons such as corn, barley, wheat, rye, rice, etc. It is also possible to use advantageously the promoter of the lpt2 or lpt1 gene (WO 95/15389, WO 95/23230) or the promotors described in WO 99/16890 (promoters of the hordein gene, the glutelin gene, the oryzin gene, the prolamin gene, the gliadin gene, the glutelin gene, the zein gene, the kasirin gene and the secalin gene).

Examples of tuber-, storage root- or root-specific promoters are the patatin promoter class I (B33) and the potato cathepsin D inhibitor promoter.

Examples of leaf-specific promoters are the cytosolic FBPase promoter from potato (WO 97/05900), the rubisco (ribulose-1,5-bisphosphate carboxylate) SSU (small subunit) promoter and the potato ST-LSI promoter (Stockhaus et al. (1989) EMBO J 8:2445-2451).

Examples of flower-specific promoters are the phytoene synthase promoter (WO 92/16635) and the promoter of the P-rr gene (WO 98/22593).

Examples of anther-specific promoters are the 5126 promoter (US 5,689,049, US 5,689,051), the glob-1 promoter and the g-zein promoter.

Further promoters suitable for expression in plants have been described (Rogers et al. (1987) Meth in Enzymol 153:253-277; Schardl et al. (1987) Gene 61:1-11; Berger et al. (1989) Proc Natl Acad Sci USA 86:8402-8406).

The site of vitamin E, plastoquinone and/or carotenoid biosynthesis in plants is, inter alia, the leaf tissue so that leaf-specific expression of the inventive nucleic acids encoding a prenyltransferase makes sense. However, this is not limiting, since the expression may also take place in a tissue-specific manner in all of the remaining parts of the plant, in particular in fat-containing seeds.

A further preferred embodiment therefore relates to a seed-specific expression of the inventive nucleic acids encoding a prenyltransferase.

In addition, a constitutive expression of exogenous prenyltransferase genes is advantageous. On the other hand, however, an inducible expression may also be desirable.

In the method of the invention, particular preference is given to constitutive and seed-specific promoters.

The efficacy of the expression of the transgenically expressed prenyltransferase gene may be determined, for example, *in vitro* by shoot-meristem propagation.

Moreover, an expression of the prenyltransferase gene, which has been modified with respect to type and level, and the effect thereof on the rate of vitamin E biosynthesis may be assayed using test plants in greenhouse experiments.

An expression cassette is preferably prepared by fusing a suitable promoter to an above-described nucleic acid encoding a prenyltransferase and, preferably, to a nucleic acid which has been inserted between promoter and nucleic acid sequence and which codes for a chloroplast-specific transit peptide and also to a polyadenylation signal according to familiar recombination and cloning techniques as described, for example, in T. Maniatis, E.F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and in T.J. Silhavy, M.L. Berman and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and in Ausubel, F.M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

Particular preference is given to inserted nucleic acid sequences which ensure targeting in the plastids.

It is also possible to use expression cassettes whose nucleic acid sequence codes for a prenyltransferase fusion protein, one part of the fusion protein being a transit peptide which controls translocation of the polypeptide. Preference is given to chloroplast-specific transit peptides which, after translocation of prenyltransferase into the chloroplasts, are enzymatically cleaved off the prenyltransferase part.

Particular preference is given to the transit peptide which is derived from the *Nicotiana tabacum* plastid transketolase or from another transit peptide (e.g. the transit peptide of the rubisco small subunit or of ferredoxin NADP oxidoreductase and also of isopentenyl pyrophosphate isomerase-2) or from its functional equivalent.

Particular preference is given to nucleic acid sequences of three cassettes of the plastid transit peptide of the tobacco plastid transketolase in three reading frames as KpnI/BamHI fragments with an ATG codon in the NcoI cleavage site:

pTP09

KpnI_GGTACCATGGCGTCTTCTTCTTCTCTCACTCTCTCTCAAGCTATCCTCTCTCGTTCTG
TCCCTCGCCATGGCTCTGCCTCTTCTTCTCAACTTTCCCCTTCTTCTCT-
CACTTTTTCCGGCCTTAAATCCAATCCCAATATCACCACCTCCCGCCGCCG-
TACTCCTTCCTCCGCCGCCGCCGCCGCCGTCGTAAGGTCACCGGCGATTCGTGCCT-
CAGCTGCAACCGAAACCATAGAGAAAACTGAGACTGCGGGATCC_BamHI

pTP10

KpnI_GGTACCATGGCGTCTTCTTCTTCTCTCACTCTCTCTCAAGCTATCCTCTCTCGTTCTG
TCCCTCGCCATGGCTCTGCCTCTTCTTCTCAACTTTCCCCTTCTTCTCT-
CACTTTTTCCGGCCTTAAATCCAATCCCAATATCACCACCTCCCGCCGCCG-
TACTCCTTCCTCCGCCGCCGCCGCCGCCGTCGTAAGGTCACCGGCGATTCGTGCCT-
CAGCTGCAACCGAAACCATAGAGAAAACTGAGACTGCGCTGGATCC_BamHI

pTP11

KpnI_GGTACCATGGCGTCTTCTTCTTCTCTCACTCTCTCTCAAGCTATCCTCTCTCGTTCTG
TCCCTCGCCATGGCTCTGCCTCTTCTTCTCAACTTTCCCCTTCTTCTCT-
CACTTTTTCCGGCCTTAAATCCAATCCCAATATCACCACCTCCCGCCGCCG-
TACTCCTTCCTCCGCCGCCGCCGCCGCCGTCGTAAGGTCACCGGCGATTCGTGCCT-
CAGCTGCAACCGAAACCATAGAGAAAACTGAGACTGCGGGGATCC_BamHI

Further examples of a plastid transit peptide are the transit peptide of the plastid isopentenyl pyrophosphate isomerase-2 (IPP-2) from *Arabidopsis thaliana* and the transit peptide of the ribulose bisphosphate carboxylase small subunit (rbcS) from pea (Guerineau, F, Woolston, S, Brooks, L, Mullineaux, P (1988) An expression cassettte for targeting foreign proteins into the chloroplasts. Nucl. Acids res. 16: 11380).

Plant genes of the invention which encode a plant prenyltransferase may already contain the nucleic acid sequence which encodes a plastid transit peptide. In this case, a further transit peptide is not required. For example, the sequence of the *Arabidopsis thaliana* prenyltransferase of the invention (SEQ. ID. NO. 2) already contains a transit peptide.

The nucleic acids of the invention may be prepared synthetically or obtained naturally or comprise a mixture of synthetic and natural nucleic acid components and may also be composed of

various heterologous gene sections of various organisms.

As described above, preference is given to synthetic nucleotide sequences with codons which are preferred by plants. These codons which are preferred by plants may be determined from codons which have the highest frequency in proteins and which are expressed in most of the interesting plant species.

When preparing an expression cassette, it is possible to manipulate various DNA fragments in order to obtain a nucleotide sequence which expediently can be read in the correct direction and is provided with a correct reading frame. The DNA fragments may be linked to one another by attaching adaptors or linkers to said fragments.

Expediently, the promoter and terminator regions may be provided in the direction of transcription with a linker or polylinker which contains one or more restriction sites for inserting this sequence. Normally, the linker has from 1 to 10, usually from 1 to 8, preferably from 2 to 6, restriction sites. Generally, the linker is within the regulatory regions less than 100 bp, frequently less than 60 bp, but at least 5 bp, in length. The promoter may be both native or homologous and non-native or heterologous to the host plant. The expression cassette preferably includes in the 5'-3' direction of transcription the promoter, a coding nucleic acid sequence or a nucleic acid construct and a region for transcriptional termination. Various termination regions can be exchanged with one another randomly.

An example of a terminator is the ocs terminator (Gielen, J, de Beuckeleer, M, Seurinck, J, Debroek, H, de Greve, H, Lemmers, M, van Montagu, M, Schell, J (1984) The complete sequence of the TL-DNA of the Agrobacterium tumefaciens plasmid pTiAch5. EMBO J. 3: 835-846).

It is furthermore possible to use manipulations which provide appropriate restriction cleavage sites or which remove excess DNA or restriction cleavage sites. In those cases for which insertions, deletions or substitutions such as, for example, transitions and transversions are suitable, *in vitro* mutagenesis, primer repair, restriction or ligation can be used.

In suitable manipulations such as, for example, restriction, "chewing-back" or filling-in of protruding ends to form blunt ends, complementary fragment ends may be provided for ligation.

Preferred polyadenylation signals are plant polyadenylation signals, preferably those which correspond essentially to T-DNA polyadenylation signals from *Agrobacterium tumefaciens*, in particular of the T-DNA gene 3 (octopine synthase) of the Ti plasmid pTiACH5 (Gielen et al., EMBO

J. 3 (1984), 835 ff) or functional equivalents.

The invention further relates to the use of the above-described nucleic acids encoding a prenyltransferase or of the above-described nucleic acid constructs or of the prenyltransferase for preparing transgenic organisms, in particular plants.

Preferably, these transgenic plants have an increased vitamin E, plastoquinone and/or carotenoid content compared to the wild type.

Therefore, the invention further relates to the use of the nucleic acids of the invention or of the nucleic acid constructs of the invention for increasing the vitamin E, plastoquinone and/or carotenoid content in organisms which, as wild type, are capable of producing vitamin E.

It is known that plants with a high vitamin E content have increased resistance to abiotic stress. Abiotic stress means, for example, cold, frost, drought, heat and salt.

Therefore, the invention furthermore relates to the use of the nucleic acids of the invention for preparing transgenic plants which have increased resistance to abiotic stress compared to the wild type.

The above-described proteins and nucleic acids may be used for producing vitamin E, plastoquinone and/or carotenoids in transgenic plants.

The transfer of foreign genes into the genome of an organism, in particular of a plant, is referred to as transformation.

For this purpose, methods known per se for transforming plants and regenerating plants from plant tissues or plant cells can be used, in particular in plants, for transient or stable transformation.

Suitable methods for the transformation of plants are the protoplast transformation by polyethylene glycol-induced DNA uptake, the biolistic method using the gene gun – also known as particle bombardment method, electroporation, the incubation of dry embryos in a DNA-containing solution, microinjection and the above-described Agrobacterium-mediated gene transfer. Said methods are described, for example, in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press (1993), 128-143 and in Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42

(1991), 205-225).

The construct to be expressed is preferably cloned into a vector which is suitable for transforming *Agrobacterium tumefaciens*, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984), 8711) or, particularly preferably, pSUN2 (WO 02/00900).

Accordingly, the invention furthermore relates to vectors containing the above-described nucleic acids, nucleic acid constructs or expression cassettes.

Agrobacteria which have been transformed with an expression cassette can be used in a known manner for the transformation of plants, for example by bathing injured leaves or leaf sections in an agrobacteria solution and then culturing them in suitable media.

Apart from in plants, the expression cassette may also be used for transforming bacteria, in particular cyanobacteria, mosses, yeasts, filamentous fungi and algae.

Genetically modified plants, also referred to as transgenic plants hereinbelow, are preferably prepared by cloning the fused expression cassette which expresses a prenyltransferase into a vector, for example pBin19, which is suitable for transforming *Agrobacterium tumefaciens*.

Agrobacteria which have been transformed with such a vector may then be used in a known manner for the transformation of plants, in particular of crop plants, for example by bathing injured leaves or leaf sections in an agrobacteria solution and then culturing them in suitable media.

The transformation of plants by agrobacteria is described, inter alia, in F.F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38. Transgenic plants which contain a gene for expression of a nucleic acid encoding a prenyltransferase, which has been integrated into the expression cassette, can be regenerated in a known manner from the transformed cells of the injured leaves or leaf sections.

A host plant is transformed with a prenyltransferase-encoding nucleic acid by incorporating an expression cassette as insertion into a recombinant vector whose vector DNA comprises additional functional regulatory signals, for example sequences for replication or integration. Suitable vectors are described inter alia, in Methods in Plant Molecular Biology and Biotechnology (CRC Press), chapter 6/7, pp. 71-119 (1993).

By way of example, the plant expression cassette may be incorporated into a derivative of the transformation vector pBin–19 with 35s promoter (Bevan, M., Nucleic Acids Research 12: 8711–8721 (1984)).

Using the above-cited recombination and cloning techniques, it is possible to clone the expression cassettes into suitable vectors which make possible their propagation, for example in *E. coli*. Suitable cloning vectors are, inter alia, pBR322, pUC series, M13mp series and pACYC184. Particularly suitable are binary vectors which can replicate both in *E. coli* and in agrobacteria.

The invention therefore further relates to the use of the above-described nucleic acids, of the above-described nucleic acid constructs, in particular of the expression cassettes, for preparing genetically modified plants or for transforming plants, plant cells, plant tissues or parts of plants.

The use is preferably aimed at increasing the vitamin E, plastoquinone and/or carotenoid content of the plant or parts of the plant.

Depending on the choice of the promoter, expression may take place specifically in the leaves, in the seeds, in petals or in other parts of the plant.

Accordingly, the invention further relates to a method for preparing genetically modified organisms by introducing an above-described nucleic acid or an above-described nucleic acid construct into the genome of the starting organism.

The invention further relates to the genetically modified organisms, the genetic modification increasing the activity of a prenyltransferase compared to a wild type and the prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

As illustrated above, the prenyltransferase activity is increased compared to the wild type preferably by increasing the gene expression of a nucleic acid encoding a prenyltransferase.

In a further preferred embodiment, gene expression of a nucleic acid encoding a prenyltransferase is increased, as illustrated above, by introducing nucleic acids encoding a prenyltransferase into the organism and thus by overexpressing nucleic acids encoding a prenyltransferase.

As mentioned above, preferred transgenic organisms contain at least one exogenous or transgenic or at least two endogenous prenyltransferase genes according to the invention.

In a preferred embodiment, the starting organisms and, accordingly, also the genetically modified organisms used as organisms and for producing organisms having an increased vitamin E, plastoquinone and/or carotenoid content in comparison with the wild type are, as mentioned above, photosynthetically active organisms such as, for example, cyanobacteria, mosses, algae or plants, particularly preferably plants.

Such transgenic plants, their propagation material and their plant cells, plant tissues or parts are a further subject of the present invention.

Genetically modified or transgenic organisms means the corresponding transformed starting organisms.

Preferred cyanobacteria are cyanobacteria of the genus *Synechocystis*.

Preferred algae are green algae such as, for example, algae of the genus *Haematococcus*, *Phaedactylum tricornatum*, *Volvox* or *Dunaliella*.

Preferred plants are, as illustrated above, tagetes, sunflower, Arabidopsis, tobacco, red pepper, soybean, tomato, egg-plant, paprika, carrot, potato, corn, lettuce and cabbage species, cereals, alfalfa, oats, barley, rye, wheat, triticale, millet, rice, lucerne, flax, cotton, hemp, rape, canola, sugar beet, sugar cane, nut and vine species or wood plants such as, for example, aspen or yew.

Particular preference is given to *Arabidopsis thaliana*, *Tagetes erecta*, *Brassica napus*, *Nicotiana tabacum*, sunflower, canola, potato, soybean and further oilseed species.

As described above, the genetically modified organisms, in particular plants, can be used for producing vitamin E, plastoquinone and/or carotenoids.

Genetically modified plants of the invention, which have an increased vitamin E, plastoquinone and/or carotenoid content and which can be consumed by humans and animals, can also be used as food- or feedstuffs or as feed and food supplements, for example directly or after processing known per se. The genetically modified organisms may furthermore be used for producing vitamin E, plastoquinone and/or carotenoid -containing extracts of said organisms and/or for

producing feed and food supplements.

Concerning the carotenoids, the methods according to the invention leads to higher flux into the carotenoids, leading to an increase of the total carotenoid content. Depending on the ability of the organism to produce certain carotenoids and the possibility of genetically modifying the organisms in order to produce certain carotenoids, the content of different carotenoids will be increased. An increased carotenoid content normally means an increased total carotenoid content. However, an increased carotenoid content also means, in particular, a modified content of at least one of the preferred carotenoids, without the need for an inevitable increase in the total carotenoid content.

Preferred carotenoids are lycopin, β-carotene, lutein, zeaxanthin, astaxanthin, violaxanthin, antheraxanthin, neoxanthin, or capsorubin and their esters.

Vitamin E means at least on chemical compound with vitamin E activity selected from the group α–tocopherol, β–tocopherol, γ–tocopherol, δ–tocopherol, α–tocotrienol, β–tocotrienol, γ–tocotrienol and δ–tocotrienol.

An increased vitamin E content normally means an increased total vitamin E content. However, an increased vitamin E content also means, in particular, a modified content of the above-described 8 compounds with vitamin E activity, without the need for an inevitable increase in the total vitamin E content.

Vitamin E is the preferred product of the method according to the invention.

The method according to the invention using the prenyltransfrases according to the invention leads to an increase of the vitamin E content compared to the wild type. Compared to the use of phytyltransferases of the prior art, using the prenyltransfrases according to the invention, the increase of the tocotrienol compounds is higher than the increase of tocopherol compounds.

The invention further relates to:

I. A polynucleotide that encodes a polypeptide of SEQ NO 1.

II. A polynucleotide comprising at least 30 contiguous bases of SEQ ID NO 1.

III. A polynucleotide having at least 60 % sequence identity to SEQ ID NO 1, wherein the identity is based on the entire coding sequence and is determined by BLAST 2.0 using

default parameters.

IV. A polynucleotide having at least 60 % sequence identity to SEQ NO 1, wherein the % sequence identity is based on the entire sequence and is determined by GAP using default parameters.

V. A polynucleotide which selectively hybridizes, under stringent conditions and a wash in 2 X SSC at 50 °C, to a hybridization probe derivable from the polynucleotide sequence as set forth in SEQ ID NO 1, or from the genomic sequence represented by SEQ ID NO 1.

VI. A polynucleotide complementary to a polynucleotide of claim V.

VII. The polynucleotide of claim I, wherein the tocotrienol/plastoquinone prenyltransferase polynucleotide is from glycine max, maize, soybean, rice and Arabidopsis thaliana.

VIII. The polynucleotide of claim I encoding a polypeptide, in which 2-metyl-6-geranygeranyl-plastoquinol or 2-methyl-6-solanesyl-plastoquinol is modified.

IX. A vector comprising at least one polynucleotide of claim I.

X. An expression cassette comprising at least one polynucleotide of claim I operably linked to a promoter, wherein the polynucleotide is in sense or antisense orientation.

XI. A host cell which is introduced with at least one expression cassette of claim X.

XII. The host cell of claim XI that is a plant cell.

XIII. A transgenic plant comprising at least one expression cassette of claim XI.

XIV. The transgenic plant of claim XIII, wherein the plant is corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, millet Arabidopsis thaliana, tomato, Brassica, vegetables, peppers, potatoes, apples, spinach or lettuce.

XV. A seed from the transgenic plant of claim XIV.

XVI. The seed of claim XV, wherein the seed is from corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cooton, rice, barley, millet Arabidopsis thaliana, tomato, Brassica,

vegetables, peppers, potatoes, apples, spinach or lettuce.

XVII. An isolated protein comprising a member selected from the group consisting of:

a) a polypeptide comprising at least 10 contigous amino acids of SEQ ID NO 2,

b) a polypeptide which is a plant tocotrienol/tocopherol prenyltransferase,

c) a polypeptide comprising at least 55 % sequence identity to SEQ ID NO 2, wherein the sequence identity is based on the entire sequence and is determined by BLAST 2.0 using default paramters and has at least one epitope in common with a prenyltransferase.

d) a polypeptide encoded by a polynucleotide selected from SEQ ID NO 1,

e) a polypeptide of SEQ ID NO 2.

XVIII. The protein of claim XVII, wherein the polypeptide is catalytically active.

XIX. A ribonucleic acid sequence encoding the protein of claim XVIII.

XX. A method for modulating the level of prenyltransferase protein in a plant, comprising:
a) stably transforming a plant cell with a prenyltransferase polynucleotide operably linked to a promoter, wherein the polynucleotide is in sense or antisense orientation
b)growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the polynucleotide for a time sufficient to modulate the level of prenyltransferase protein in the plant.

XXI. The method of claim XX, wherein the prenyltransferase polynucleotide is selected from SEQ ID NO 1.

XXII. The method of claim XX, wherein the plant is corn, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, millet Arabidopsis thaliana, tomato, Brassica, vegetables, peppers, potatoes, apples, spinach or lettuce.

XXIII. The method of claim XX, wherein prenyltransferase is increased.

XXIV. The method of claim XX, wherein prenyltransferase is decreased.

XXV. A method for modulating the level of tocotrienol in a plant, comprising:
a) stably transforming a plant cell with a prenyltransferase polynucleotide operably linked to a promoter, wherein the polynucleotide is in sense or anti-sense orientation,
b) growing the plant cell under plant growing conditions to produce a regenerated plant

capable of expressing the polynucleotide for a time sufficient to modulate level of tocopherol in the plant.

XXVI. A method for modulating the level of plastoquinol in a plant, comprising:
a) stably transforming a plant cell with a prenyltransferase polynucleotide operably linked to a promoter, wherein the polynucleotide is in sense or anti-sense orientation.
b) growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the polynucleotide for a time sufficient to modulate level of plastoquinol in the plant.

XXVII. A method for modulating the level of carotenoids in a plant, comprising:
a) stably transforming a plant cell with a prenyltransferase polynucleotide operably linked to a promoter, wherein the polynucleotide is in sense or anti-sense orientation.
b) growing the plant cell under plant growing conditions to produce a regenerated plant capable of expressing the polynucleotide for a time sufficient to modulate level of carotenoid in the plant.

XXVIII. The method of claims XXV, XXVI, XXVII, wherein the prenyltransferase polynucleotide is selected from SEQ ID NO 1.

The present invention further relates to the use of a plant prenyltransferase as target for the identification of herbicidal agents and methods of identifying herbicidal agents which inhibit plant prenyltransferase comprising the following steps:

i. incubating, with at least one candidate compound, a plant prenyltransferase under conditions allowing the binding of the candidate compound to the plant prenyltransferase polypeptide; and

ii. selecting, by step ii), at least a candidate compound which binds to the plant prenyltransferase of step i); or

iii. selecting, by step iii), at least a candidate compound which reduces or blocks the activity of the plant prenyltransferase of step i); or

iv. selecting, by step iv), at least a candidate compound which inhibits or decreases transcription, translation or expression of the plant prenyltransferase of step i).

Some of the terms used further on in the description are defined at this point.

"Affinity tag": this denotes a peptide or polypeptide whose coding nucleic acid sequence can be fused to the sequence encoding the plant prenyltransferase, either directly or using a linker, by customary cloning techniques. The affinity tag serves to isolate the recombinant plant prenyltransferase by means of affinity chromatography. The abovementioned linker can optionally comprise a protease cleavage site (for example for thrombin or factor Xa), whereby the affinity tag can be cleaved off from the plant prenyltransferase, as required. Examples of customary affinity tags are the "his-tag", for example from Quiagen, Hilden, "strep-tag", "myc-tag" (Invitrogen, Carlsberg), New England Biolab's tag which consists of a chitin binding domain and an intein, and what is known as the CBD-tag from Novagen.

"Herbicidal agents" are agents against non cultural plants

"Enzymatic activity/activity assay": the term enzymatic activity describes the ability of an enzyme to convert a substrate into a product. In this context, both the natural substrate of the enzyme and a synthetic modified analog of the natural substrate can be used. The enzymatic activity can be determined in what is known as an activity assay via the increase in the product, the decrease in the starting material, the decrease or increase in a specific cofactor, or a combination of at least two of the aforementioned parameters as a function of a defined period of time. If the enzyme catalyzes a reversible reaction, both the starting material and the product may be employed as substrate in the activity assay in question.

"Functional equivalents" in the present context describe nucleic acid sequences which hybridize under standard conditions with the nucleic acid sequence encoding the prenyltransferase or portions of the nucleic acid sequence encoding the prenyltransferase, and which are capable of bringing about the expression of an enzymatically active plant prenyltransferase in a cell or an organism.

It is advantageous to use short oligonucleotides of a length between 10 to 50bp, preferably 15-40bp, for example of the conserved or other regions, which can be determined via comparisons with other related genes in a manner known to the skilled worker for the hybridization. Alternatively, it is also possible to use longer fragments of the nucleic acids according to the invention or the complete sequences for the hybridization. These standard conditions vary depending on the nucleic acid used, viz. oligonucleotide, longer fragment or complete sequence, or depending on which type of nucleic acid, viz. DNA or RNA, is being used for the hybridization. Thus, for example, the melting temperatures for DNA:DNA hybrids are approx. 10°C lower than those of

DNA:RNA hybrids of equal length. Suitable hybridization conditions are described above.

A functional equivalent is furthermore also understood as meaning, in particular, natural or artificial mutations of the relevant nucleic acid sequences of the plant prenyltransferase and their homologs from other organisms which make possible the expression of the enzymatically active plant prenyltransferase in a cell or an organism.

Thus, the scope of the present invention also extends to, for example, those nucleotide sequences which are obtained by modification of the nucleic acid sequence of a prenyltransferase. The purpose of such a modification can be, for example, the insertion of further cleavage sites for restriction enzymes, the removal of excess DNA, or the addition of further sequences. Proteins which are encoded via said nucleic acid sequences should still maintain the desired functions, despite the deviating nucleic acid sequence.

The term functional equivalent may also refer to the protein encoded by the nucleic acid sequence in question. In this case, the term functional equivalent describes a protein whose amino acid sequence is up to a specific percentage identical with that of the prenyltransferase.

Functional equivalents thus encompass naturally occurring variants of the sequences described herein, and also artificial, for example chemically synthesized, nucleic acid sequences adapted to the codon usage, or the amino acid sequences derived therefrom.

In general, it can be said that functional equivalents independently of the amino acid sequence in question (encoded by a corresponding nucleic acid sequence) have in each case the enzymatic activity of a prenyltransferase.

"Reporter genes" encode readily quantifiable proteins. Using these genes, an assessment of transformation efficacy or of the site or time of expression can be made via growth, fluorescence, chemoluminescence, bioluminescence or resistance assay or via photometric measurement (intrinsic color) or enzyme activity. Very especially preferred in this context are reporter proteins (Schenborn E, Groskreutz D. Mol. Biotechnol. 1999; 13(1):29-44) such as the "green fluorescence protein" (GFP) (Gerdes HH and Kaether C, FEBS Lett. 1996; 389(1):44-47; Chui WL et al., Curr. Biol. 1996, 6:325-330; Leffel SM et al., Biotechniques. 23(5):912-8, 1997), chloramphenicol acetyl transferase, a luciferase (Giacomin, Plant Sci. 1996, 116:59-72; Scikantha, J. Bact. 1996, 178:121; Millar et al., Plant Mol. Biol. Rep. 1992 10:324-414), and luciferase genes, in general β-galactosidase or β-glucuronidase (Jefferson et al., EMBO J. 1987, 6, 3901-3907), the Ura3 gene, the Ilv2 gene, the 2–desoxyglucose–6–phosphate phosphatase gene, β-lactamase gene, the neomycin phosphotransferase gene, the hygromycin phosphotransferase

gene, or the BASTA (= gluphosinate) resistance gene.

"Significant decrease": referring to the enzymatic activity, is understood as meaning the decrease in the enzymatic activity of the enzyme incubated with a candidate compound in comparison with the activity of an enzyme not incubated with the candidate compound, which lies outside an error in measurement.

"Substrate": Substrate is the compound which is recognized by the enzyme in its original function and which is converted into a product by means of a reaction catalyzed by the enzyme.

In one embodiment, the present invention encompasses a method for identifying herbicidal agents comprising the following steps:

i. incubating, with at least one candidate compound, a plant prenyltransferase under conditions allowing the binding of the candidate compound to the plant prenyltransferase ; and

ii. selecting, by step ii), at least a candidate compound which binds to the plant prenyltransferase of step i); or

iii. selecting, by step iii), at least candidate compound which reduces or blocks the activity of the plant prenyltransferase of step i); or

iv. selecting, by step iv), at least candidate compound which inhibits or decreases transcription, translation or expression of the plant prenyltransferase .

Preferably, the plant prenyltransferase is encoded by a nucleic acid sequence comprising

a) a nucleic acid sequence shown in SEQ ID NO:1; or

b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO: 2 by back translation; or

c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID NO: 2, which has an identity with SEQ ID NO:2 of at least 50%, by back translation.

The functional equivalent of SEQ ID NO:2 set forth in c) has an identity of at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57% preferably at least 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, and 70% more preferably 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85% most preferably at least 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the SEQ ID NO:2.

The selection according to step ii) can be based on binding assays detecting the portein-inhibitor interactions, wherein either the candidate compound or plant prenyltransferase comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. Detection of a candidate compound, which is bound to the plant prenyltransferase can then be accomplished, for example, by direct counting of radioemmission, by scintillation counting, or by determining conversion of an appropriate Substrate to a detectable product.

Preferred example of these binding assays are Fluorescence correlation spectroscopy (FCS) (Proc. Natl. Acad. Sci. USA (1994) 11753-11575), flurescence polarization (Methods in Enzymology 246 (1995), pp. 283-300) or Flurescence Energy Transfer (FRET) (Cytometry 34, 1998, pp. 159-179; homogenous Time Resoved Fluorescence (HTRF) is preferred, if FRET is to be used).

Alternatively, binding of a candidate compound to a plant prenyltransferase can be determined without labeling either of the interactants e.g. by using a microphysiometer to detect binding of a candidate compound to the plant prenyltransferase . A microphysiometer (e.g., Cytosensor™) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric Sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a candidate compound and plant prenyltransferase (according to McConnell et al., Science 2.57, 19061912, 1992). In addition, the determining the ability of a candidate compound to bind to the plant prenyltransferase can be accomplished using a technology such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, Anal. Chem. 63, 23382345, 1991, and Szabo etal., Curr. Opin. Struct. Biol. 5,699705, 1995), a technology for studying biospecific interactions in real time, without labeling any of the interactants (e.g. BIAcore). Changes in the optical phenomeon surface plasmon resonance can be used as an indication of real-time reactions between biological molecules. Also Surface-enhanced laser desorption/ionization (SELDI) in combination with a time-of-flight mass spectrometer (MALDI-TOF) makes possible the rapid analysis of molecules on a support and can be used for analyzing protein-ligand interactions (Worral et al., (1998) Anal. Biochem. 70:750-756).

Alternatively, all of the above-mentioned methods can be based on a "competition assay", wherein a reference molecule is replaced by the candidate compound.

It is also possible to detect further potential herbicidal agents by "molecular modeling" via elucidation of the three-dimensional structure of the polypeptide according to the invention using x-ray structure analysis. The preparation of protein crystals required for x-ray structure analysis, and the corresponding measurements and subsequent evaluations of said measurements, as well as the methodology of "molecular modeling" are known to the skilled worker. In principle, an optimization via "molecular modeling" of the active ingredients identified by the abovementioned methods is also possible.

The selection according to step iii) and iv) preferably comprises testing a candidate compound in a plant prenyltransferase inhibition assay.

By preference, the selection according to step iii) herein after referred to as "In vitro assay" is based on the following steps:

a) incubating, with a candidate compound, the plant prenyltransferase in a cell free system;

b) selecting, by step b), a candidate compound which decreases the activity of plant prenyltransferase .

The enzymatic activity of the plant prenyltransferase is preferably determined in comparison to the activity of a plant prenyltransferase not incubated with the candidate compound.

In step (b), candidate compounds are selected which brought about a significant decrease in the enzymatic activity corresponding to a reduction of at least 10%, advantageously at least 20%, preferably at least 30%, especially preferably by at least 50% and very especially preferably by at least 70%, or a 100% reduction (blocking) being achieved.

Suitable substrates added to the reaction mixture in step b) for determination of enzymatic aciti-vity is geranylgeranyl pyrophosphate comprising a detectable label, such as a fluorescent, radioisotopic or chemiluminescent lable.

For determination of enzymatic activity of plant prenyltransferase in step b) of the in vitro assay a plant prenyltransferase comprising mixture (e.g. crude cell extract, partially or totally purified protein) is incubated with a suitable substrate and the conversion of the substrate or the in-

crease in the resultant product is monitored e.g. by HPLC or by measurement of fluorescence, radioactivity or chemiluminescence of the respective sample.

When a sample comprising an herbicidal agent has been identified by the method according to the invention, it is either possible to isolate the substance directly from the original samples, or else the sample can be divided into different groups, for example when it consists of a multiplicity of different components, in order to reduce the number of different substances per sample and then to repeat the method according to the invention with such a "subsample" of the original sample. Depending on the complexity of the sample, the above-described steps can be repeated several times, preferably until the sample identified in accordance with the method according to the invention only encompasses a small number of substances or only one substance. The substance identified in accordance with the method according to the invention, or derivatives thereof, is preferably formulated further so that it is suitable for use in plant breeding or in plant cell or plant tissue culture.

All of the herbicidal agents identified by the abovementioned methods can subsequently be tested for their herbicidal action in an further in-vivo activity test.

The above-mentioned embodiments of the method for identifying of herbicidal agents are preferably realized in a high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of candidate compounds can be quickly screened.

The most widely established techniques utilize 96-well, 384-well and 1536-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 µl, preferably 200 µl. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the respective well format.

Alternatively, free format assays or assays that have no physical barrier between samples, can be used as described in Jayaickreme et al. (Proc. Natl. Acad. Sci U.S.A. 19 (1994) 161418), Chelsky ("Strategies for Screening Combinatorial Libaries, First Annual Conference of The Society for Biomolecular Screening in Philadelphia, Pa. (Nov. 710, 1995)) and Salmon et al. (Molecular Diversity 2 (1996), 5763). Additionally, high throughput screening method as described in U.S. Patent 5,976,813 can be used based on a porous matrix, in which test samples are placed, one or more assay components are then placed within, on top of, or at the bottom of a matrix such as a gel, a plastic sheet, a filter, or other form of easily manipulated solid support. When samples are introduced to the porous matrix they diffuse sufficiently slowly, such that the assays

tan be performed without the test samples running together.

It may be desirable for HTS to immobilize either the plant prenyltransferase or the candidate compound to facilitate separation of bound and unbound forms of one or both of the interactants, as well as to accommodate automation of the assay. Thus, either the plant prenyltransferase or the candidate compound is preferably bound to a solid support. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, Silicon Chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the plant prenyltransferase or candidate compound to a solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached respectively to the plant prenyltransferase or candidate compound and the solid support. candidate compounds are preferably bound to the solid support in an array, so that the location of individual candidate compounds tan be tracked. Binding of a candidate compound to a plant prenyltransferase. can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

All of the herbicidal agents identified by the abovementioned methods further designated as "identified compounds" are subject matter of the present invention. Preferably, they have a molecular weight below 1000g/mol, preferably 500g/mol, more preferably 400g/mol and most preferably 300g/mol. The identified compounds further exhibit a Ki-value below 1mM, preferably 1 μM, more preferably 0.1 μM and most preferably 0.01 μM.

The identified compounds may be: expression libraries, for example cDNA expression libraries, peptides, proteins, nucleic acids, antibodies, small organic substances, hormones, PNA(s) or the like (Milner, Nature Medicin 1 (1995), 879–880; Hupp, Cell. 83 (1995), 237–245; Gibbs, Cell. 79 (1994), 193–198 and references cited therein). They may be chemically synthesized substances or substances produced by microorganisms and can be present for example in cell extracts or, for example, plants, animals or microorganisms. The reaction mixture can be a cell-free extract or comprise a cell or cell culture. Suitable methods are known to the skilled worker and are described generally for example in Alberts, Molecular Biology the cell, 3rd Edition (1994), for example Chapter 17. For example, the substances mentioned can be added to the reaction mixture or the culture medium or injected into the cells or sprayed onto a plant.

The identified compounds may also be present in the form of their agriculturally useful salts. Suitable salts among agriculturally useful salts are mainly the salts of those cations or the acid addition salts of those acids whose cations, or anions, respectively, do not adversely affect the

herbicidal action of the identified compound.

All of the identified compounds - if they comprise asymmetrically substituted α-carbon atoms - exist either as racemates, enantiomer mixtures or as pure enantiomers and - if they have chiral substituents - may also exist as diastereomer mixtures.

The invention therefore furthermore relates to processes for the preparation of the herbicidal composition, which comprises

a) selection of an identified compound; and

b) formulating the identified compound, or an agriculturally useful salt of the identified compound identified via (a), with suitable adjuvants.

The identified compounds according to the invention in step a) can be formulated for example in the form of directly sprayable aqueous solutions, powders, suspensions, also highly concentrated aqueous, oily or other suspensions or suspoemulsions or dispersions, emulsions, oil dispersions, pastes, dusts, compositions for spreading, or granules, and applied by spraying, atomizing, dusting, spreading or pouring. The use forms depend on the intended purposes and the nature of the identified compound used; in any case, they should ensure the finest possible distribution of the identified compounds according to the invention.

For the preparation of emulsions, pastes or aqueous or oil-containing dispersions, the substances and/or compositions the identified compounds as such or can be dissolved or dispersed in an oil or solvent, it being possible to add further formulation auxiliaries for homogenization. However, it is also possible to prepare liquid or solid concentrates which are composed of identified compound and, if appropriate, solvent or oil and optionally further auxiliaries, and these concentrates are suitable for dilution with water. Materials which may be mentioned in this context are emulsion concentrates (EC, EW), suspensions (SC), soluble concentrates (SL), pastes, pellets, wettable powders or granules, it being possible for the solid formulations to be either soluble or dispersible (wettable) in water. Moreover, such powders or granules or tablets may additionally be provided with a solid coating which prevents abrasion or an unduly early release of the identified compound.

The term auxiliaries is understood as meaning, in principle, the following classes of substances: antifoams, thickeners, wetters, stickers, dispersants or emulsifiers, bactericides and thixotropic agents. The skilled worked is familiar with the meaning of the abovementioned agents; they are

described, for example, in.

SLs, EWs and ECs can be prepared by simply mixing the constituents in question; powders can be prepared via mixing or grinding in specific types of mills (for example hammer mills). SCs and SEs are usually prepared by wet milling, it being possible to prepare an SE from an SC by adding an organic phase comprising further auxiliaries or identified compounds. The preparation is known. Granules, for example coating granules, impregnated granules and homogenous granules, can be prepared by binding the identified compounds to solid carriers. The skilled worker is familiar with a multiplicity of solid carriers which are suitable for granules according to the invention, for example mineral earths such as silicas, silica gels, silicates, talc, kaolin, limestone, lime, chalk, bole, loess, clay, dolomite, diatomaceous earth, calcium sulfate, magnesium sulfate, magnesium oxide, ground synthetic materials, fertilizers such as ammonium sulfate, ammonium phosphate, ammonium nitrate, ureas, and products of vegetable origin such as cereal meal, tree bark meal, wood meal and nutshell meal, cellulose powders or other solid carriers. The skilled worker is familiar with details of the preparation; they are stated, for example, in the following publications: US 3,060,084, EP-A 707445 (for liquid concentrates), Browning, "Agglomeration", Chemical Engineering, Dec. 4, 1967, 147-48, Perry's Chemical Engineer's Handbook, 4th Ed., McGraw-Hill, New York, 1963, pages 8-57 and ff. WO 91/13546, US 4,172,714, US 4,144,050, US 3,920,442, US 5,180,587, US 5,232,701, US 5,208,030, GB 2,095,558, US 3,299,566, Klingman, Weed Control as a Science, John Wiley and Sons, Inc., New York, 1961, Hance et al., Weed Control Handbook, 8th Ed., Blackwell Scientific Publications, Oxford, 1989 and Mollet, H., Grubemann, A., Formulation technology, Wiley VCH Verlag GmbH, Weinheim (Federal Republic of Germany), 2001.

The skilled worker is familiar with a multiplicity of inert liquid and/or solid carriers which are suitable for the formulations according to the invention, such as, for example, liquid additives such as mineral oil fractions of medium to high boiling point, such as kerosene or diesel oil, furthermore coal tar oils and oils of vegetable or animal origin, aliphatic, cyclic and aromatic hydrocarbons, for example paraffin, tetrahydronaphthalene, alkylated naphthalenes or their derivatives, alkylated benzenes or their derivatives, alcohols such as methanol, ethanol, propanol, butanol, cyclohexanol, ketones such as cyclohexanone, or strongly polar solvents, for example amines such as N-methylpyrrolidone, or water.

The skilled worker is familiar with a multiplicity of surface-active substances (surfactants) which are suitable for the formulations according to the invention, such as, for example, the alkali, alkaline earth or ammonium salts of aromatic sulfonic acids, for example lignin sulfonic acid, phenol sulfonic acid, naphthalene sulfonic acid and dibutylnaphthalenesulfonic acid, and of fatty acids, alkyl sulfonates, alkylaryl sulfonates, alkyl sulfates, lauryl ether sulfates and fatty alcohol

sulfates, and salts of sulfated hexadecanols, heptadecanols and octadecanols, and of fatty alcohol glycol ethers; condensates of sulfonated naphthalene and its derivatives with formaldehyde, condensates of naphthalene or of the naphthalene sulfonic acids with phenol and formaldehyde, polyoxyethylene octylphenol ether, ethoxylated isooctylphenol, octylphenol or nonylphenol, alkylphenyl or tributylphenyl polyglycol ether, alkylaryl polyether alcohols, isotridecyl alcohol, fatty alcohol/ethylene oxide condensates, ethoxylated castor oil, polyoxyethylene alkyl ethers or polyoxypropylenealkyl ethers, lauryl alcohol polyglycol ether acetate, sorbitol esters, lignin-sulfite waste liquors or methylcellulose.

Powders, dusts and materials for spreading, being solid carriers, can be prepared advantageously by mixing or concomitantly grinding the active substances with a solid carrier.

The concentrations of the identified compounds in the ready-to-use preparations can be varied within wide limits and depends on the nature of the formulation in question.

The herbicidal compositions, or the identified compounds, can be applied in curativ.

The applications of identified compounds (= substances and/or compositions) amount to from 0.001 to 3.0, preferably 0.01 to 1.0 kg/ha active substance, depending on the aim of the control measures, the season, the target plants and the stage of growth.

The invention is illustrated by the examples below, but is not limited thereto:

Example 1

Cloning of prenyltransferase from Arabidopsis thaliana

The prenyltransferase cDNA was amplified from an *Arabidopsis* Matchmaker cDNA library (Clontech, Germany) by using the primers *PQ5* (SEQ ID NO: 3) and *PQ3* (SEQ ID NO: 4). The purified PCR-product was cloned into *E.coli* expression vector pBADTOPO. Two independent clones, PQBAD1 and PQBAD2, were sequenced and no differences between them could be detected. The 1161 bp coding sequence of the prenyltransferase (Fig. 2; SEQ ID NO: 1) codes for a deduced protein of 386 amino acids and ~42.84 KDa (Fig.3; SEQ ID NO: 2).

1.1 Cloning procedures

A 1193 bp-fragment, representing the 1161 bp coding sequence of the prenyltransferase cDNA and additional 32 bp, was amplified from 1 µg of an *Arabidopsis* Matchmaker cDNA library (Clontech, Germany) using the primers *PQ5* (5'-

AAGCTTCTGGAGCTGTCGATCTCACAATCACCGCGT-3'; SEQ ID NO 3) and *PQ3* (5'-CATATCGTCGACATGAAATTGAAAGCTAGAGGAAGG-3'; SEQ ID NO 4).

PCR with Taq-polymerase (Amersham Pharmacia Biotech, Germany) was carried out after a denaturation of 3 min at 94°C for 30 cycles (60 s at 94°C, 60 s at 60°C, 80 s at 72°C), followed by an extension at 72°C for 10 min. Thereby a *SalI*-site was generated in the 3'-non coding region of the PCR-product, as well as the start ATG-codon (to CTG) and a *SacI*-site (to GAGCTG) modified in the coding sequence for latter cloning strategies. The modification in the *SacI*-site leads to a conserved *Leu to Leu* displacement in the deduced amino acid sequence of the open reading frame.The purified PCR-product (GFX PCR and Gel Purification Kit; Amersham Pharmacia Biotech, Germany) was cloned into the *E.coli* expression vector PBADTOPO TA (Invitrogen, the Netherlands). Two independent clones in different orientations to the arabinose-promoter (PQBAD1 and PQBAD2) were sequenced and no differences between them could be detected.

The 1161bp-coding sequence was compared to the sequence of *Arabidopsis* genomic DNA (chromosome 3; P1 clone MEC18) by the GAP-program (GCG software package) and the correct exon/intron-structure could be achieved.

For the stable transformation of a BY2-tobacco cell culture, the prenyltransferase-cDNA with a correct Start-ATG was amplified from the vector PQBAD2 by proof-reading PCR with AdvanTaq-Polymerase (Clontech, Germany) using the primer pair *PQBY5* (5'-GCCCTTCCCGGGATGGAGCTGTCGATCTCACAATCA-3'; SEQ ID NO 5) and *PQBY3* (5'-TGAAATGAATTCCTAGAGGAAGGGGAATAACAGATA-3'; SEQ ID NO 6). After denaturation for 3 min at 94°C, 30 cycles (94°C for 30s/60°C for 45s/68°C for 120 s) and an extension (68°C for 5 min) were performed. The purified PCR-product was digested *SmaI/EcoRI* and cloned into the agrobacterial binary vector pC1390-35S, digested with the same enzymes. pC1390-35S is a derivative of pCambia1390 (accession no. 234307), consisting of an additional 35CaMV 35S-promoter, integrated clockwise in between the *PstI/BamHI*-sites of this vector. The resulting vector pC1390PQ-35S was transformed into BY-2 tobacco cells.

1.2 Alignments, homologies and prenyltransferase domains

In Fig. 4 a GAP-alignment between the protein-sequence of prenyltransferase and the predicted protein-sequence of MEC18.5 has been performed, also giving the parameters of the alignment. It shows clearly that the inconsistencies due to false predicted intron/exon borders are at the N-terminal end of the protein directly in front of the potential prenyltransferase domain (GAAESDDPVLDRIARFQ; aa position 75-91 of SEQ ID NO 2), the so called DDXXD-motive of

prenyltransferases, and at the C-terminal part in a range of 96 amino acids (amino acids 346 to 441 of the predicted protein). This motive of apartate (D) or glutamate(E)-residues is known to bind cations (preferentially Mg^{2+} and Mn^{2+}) to form a complex with the diphosphate moiety of isoprenoid diphosphates in the reaction center. Taken all inconsistencies of the predicted protein into account 17.9 % of the amino acids didn't match (79 aa/441 aa). Comparing the peptide-sequences of the prenyltransferase with tocopherol phytyltransferase of *Synechocystis* (SLR1736) in the same GAP-program only 38.3% identity / 48.8% similarity between them can be observed (Fig. 5). The putative prenyltransferase domain (GAAESDDPVLDRIARFQ; aa position 75 to 91 in PQP (SEQ ID NO 2)) is not recognized as a homologous sequence, as alignment by this program starts at aa 87 of SEQ ID NO 2 (IARFQ). Protein-sequences of prenyltransferase and tocopherol phytyltransferase of *Arabidopsis* were aligned by the GAP-program (Fig. 6). They have only 33.8 % identity/41.4 % similarity, revealing very low similarities in the depicted putative prenyltransferase-domain of PQP (aa 75 to 91 in SEQ ID NO 2).

Example 2

Transformation of BY-2 tobacco cell suspension culture and analysis of transformants

For stable transformation and overexpression in BY-2 tobacco cells PQP- cDNA was cloned under the control of the constitutive Cauliflower Mosaic Virus (CaMV) 35S-promoter in agrobacterial binary vector pC1390PQ-35S.

2.1 Direct DNA transfer in Agrobacterium tumefaciens

Agrobacterium tumefaciens strain GV3101 (pMP90RK) was grown for 3 days on LB-plates containing 50 µg/ml rifampicin and 25 µg/ml gentamycin at 28°C. A single colony was inoculated in 5 ml LB-medium containing the described antibiotics and grown over night at 28°C with shaking (130 rpm). For the generation of chemocompetent bacteria 4 ml over night culture were added to 100 ml LB-medium with 50 mg/ml rifampicin and 25 µg/ml gentamycin and grown as above until the culture reached an OD_{600} of 0.5 to 1.0. The culture was chilled on ice, afterwards the cell suspension was centrifuged for 5 min at 3000 *g* at 4°C. The supernatant was discarded and the cells resuspended in 1 ml 20 mM $CaCl_2$ solution (ice-cold). 0.1 ml aliquots were dispensed into prechilled Eppendorf test tubes. For transformation 1 µg of vector pC1390PQ-35S was added to the chemocompetent cells and immediately frozen in liquid nitrogen. The cells were thawed by incubating the test tube in a 37°C water bath for 5 min. Afterwards 1 ml of prewarmed LB-medium was added to the tube and the cells incubated for 4 h at 28°C with gentle shaking. Cells were isolated by a 30 sec-centrifugation, resuspended in 0.1 ml LB-medium and spread on LB-plates containing 25 µg/ml gentamycin and 20 µg/ml kanamycin. Transformed colonies appeared after 2 to 3 days. They were inoculated in LB-medium containing 50 µg/ml rifampicin, 25

µg/ml gentamycin and 20 µg/ml kanamycin and transformation verified by colony-PCR (Sambrook *et al.*, 2001).

2.2 Transformation and propagation of BY-2 cell suspension culture

A *Nicotiana tabacum* BY-2 cell suspension culture (Nagata T, Nemoto Y and Hasezawa S (1992). Tobacco BY-2 cell line as the "HeLa" cell in the cell biology of higher plants. *Int. Rev. Cytol.* **132**, 1-30) was grown in MS culture medium (1x MS salts, pH5.8; 0.2 g/l KH2PO4; 30 g/l sucrose; 0.2 mg/l 2,4-D (auxine); 1 mg/l thiamine-HCl; 0.1 g/l *myo*-inositol) at 28°C and 130 rpm in the dark. The culture was subcultured weekly by transferring 2 ml of culture into 100 ml fresh media.

For transformation, 1 ml of cell culture from day 6 *post* inoculation was mixed with 12 ml MS medium and 200 µl of *agrobacterium tumefaciens* strain GV3101 (pMP90RK), transformed with vector pC1390PQ-35S. After 4 days of incubation at 28°C in the dark cells were isolated by centrifugation (5 min at 2000 *g* and room temperature), cell pellets washed twice with culture medium containing 125 µg/ml β-bactyl (Smithkline Beecham, Germany). Afterwards the cells were transferred into 50 ml fresh culture medium containing 125 µg/ml β-bactyl and 30 µg/ml hygromycin. Reaching the stationary phase 1 ml of culture was transferred into fresh medium with additives as above. Transfer was repeated once before analysis of the transformants was performed.

Individual wild-type and transformed cell cultures were harvested and extracted at the same day.

2.3 Extraction of BY-2 cell suspension cultures

Wildtype BY-2 cells and those stably transformed with vector pC1390PQ-35S were harvested 7 days after subcultivation. Medium was removed by filtration through nylon mesh and cells lyophilized to dryness. After the determination of dry weight cells were resuspended in 35 ml of acetone. For internal standardization 40 µg of D-α-tocopherol acetate (Sigma, Germany) and 2 nmoles of echinenone, purified from *Synechocystis sp.PCC6803*, were added. Soluble compounds were extracted twice by harsh sonication on ice followed by centrifugation (10 min, 6000 *g*, 4°C). Remaining cell debris in the extracts was removed by filtration through a 0.2 µm membrane filter (Schleicher & Schuell, Germany) under vacuum. Pooled acetone phases were dried by rotary evaporation and compounds dissolved in 2 ml of chloroforme. After evaporation compounds were solubilized in 500 µl chloroforme and transferred to eppendorf tubes. Again, extracts were evaporated to dryness. Then compounds were solubilized in 50 µl chloroforme and subjected to HPLC-analysis. For the determination of lipophilic compounds wildtype BY-2 cells and three transformants were analyzed. To diminish errors in subcultivation and extraction 3

different subcultures of wildtype and transformants were cultivated at different time points and analyzed.

Analysis was three times repeated for individual cultures to diminish the effect of putative environmental factors (table 1, table 3, table 5).

Wild type and transformants grew normally. However, transformants showed a yellowish colour compared to the wild-type, indicating the presence of carotenoids. Prior to extraction dried cell cultures were internally standardized by the addition of α-tocopherol acetate (for vitamin E; fluorescence detection) and echinenone (for carotenoids; detection by absorbance).

2.4 Quantification

Decyl-plastoquinone (Sigma, Germany) was quantified spectrophotometrically at 255 nm prior and after reduction with $NaBH_4$ in absolute ethanol (ΔE_m = 15,000) (Barr R and Crane FL (1971). Quinones in algae and higher plants. *Methods Enzymol.* **13**, 372-408), 15 nmoles were used for calibration. D-α–tocopherol acetate (Sigma, Germany) was reduced with $NaBH_4$ and measured in absolute ethanol at 284 nm ($E^{1\%}_{cm}$ = 45; value given by the manufacturer). Tocopherol – and tocotrienol-standards (Merck, Germany) were quantified as described ($E^{1\%}_{cm}$ see Schuep W and Rettenmaier R (1994). Analysis of vitamin E homologs in plasma and tissue: high-performance liquid chromatography. *Methods Enzymol.* **234**, 294-302) and also analyzed in the form of their oxidized quinones after treatment with 200 µM potassium ferricyanide.

For calibration the absorption/emission peak areas of the quantified standards were analyzed by HPLC. Tocopherols and tocotrienols were detected by use of a fluorescence detector setting excitation and fluorescence emission to 290 nm and 324 nm (40 nm band width), respectively . All other standards were calibrated at their maximal absorbance.

The tocopherol content in the samples was determined by internal standardization adding 40 µg D-α–tocopherol acetate to the lyophilized bacterial pellet prior to extraction. Likewise, carotenoid content was determined by internal standardization with 2 pmoles echinenone. Quantification of echinenone-standard was performed spectrophotometrically at 461 nm (E_m =118,900). In BY-2 extracts predominantly violaxanthin, antheraxanthin, neoxanthin, β-carotene and their respective esters can be detected. To quantify the overall content of carotenoids an E_m = 133,000 was assumed.

Plastoquinone was quantified by external standardization with decyl-plastoquinone (Sigma, Germany). To identify plastoquinones in BY-2 cells, a plastoquinone-9 standard from spinach leaves was prepared by acetone-extraction (Barr and Crane, 1971). It was purified by TLC (silica

gel 60, Merck, Germany) using petroleum benzene/diethylether (7:1; v:v) (Soll J. and Schultz G. (1979). Comparison of geranylgeranyl and phytyl substituted methylquinols in the tocopherol synthesis of spinach chloroplasts. *Biochem. Biophys. Res. Commun.* **91**, 715-720).

2.5 HPLC-analysis

The HPLC-system (Waters, Germany) consisted of two 510 HPLC pumps, a 717plus autosampler, a 996 photodiode array detector monitoring UV/VIS spectra, a 474 scanning fluorescence detector and a C_{30}-reversed HPLC column (YMC Europe, Germany). Chromatograms were analyzed using the Millenium PDA software package (Waters, Germany). The column was developed at a flow rate of 1 ml/min with the solvent system A: methanol/*tert*-butylmethyl ether/water (60:12:12; v:v:v) and B: methanol/ *tert*-butylmethyl ether (50:50; v:v). A linear gradient was performed from 100 % A to 57 % A in 45 min , followed by a linear gradient to 0 % A in 35 min and an isocratic step at 0 % A for 25 min. 50 µl of chloroforme extracts were injected.

2.6 Results

With respect to vitamin E the major compound that accumulates in wild-type BY-2 cells is α-tocotrienol.The only other vitamin E compound which can be identified by its fluorescence and retention time in extracts of BY-2 cells is δ-tocopherol (table 1). For the transformants overexpressing the prenyltransferase an overall increase in α-tocotrienol levels can be observed (300-500 %; table 2; Fig. 7). In contrast, tocopherol levels are not changed significantly in transformants (88-116 %; table 2).

We also analyzed the plastoquinone-9 content of wild-type and BY-2 cells transformed with pC1390PQ-35S (table 3). Therefore we externally standardized the extracts by the utilization of decyl-plastoquinone. The retention time of plastoquinone-9 in our HPLC-system (60. min) was assayed with a plastoquinone-9 standard prepared from spinach leaves. In transformants plastoquinone-9 levels were increased to 146-169 % compared to the wild-type (table 4; Fig. 8).

Carotenoids that predominantly accumulate in BY-2 cells are xanthophylls (i.e. violaxanthin, antheraxanthin, neoxanthin and their esters) and β-carotene. For quantification of the overall carotenoid content an E_m =133, 000 was assumed.
As observed with tocotrienols and plastoquinones, carotenoid levels (table 5) were increased in transformants relative to the wild-type (209-215 %; table 6).

Summarizing BY-2 cells transformed with pC1390PQ-35S have increased levels of tocotrienols, plastoquinones and carotenoids.

We isolated a prenyltransferase of *Arabidopsis*, located at the branching point of different isoprenoid biosynthetic pathways. As tocotrienols and plastoquinones are upregulated in transformants overexpressing this gene product, one can conclude that this prenyltransferase catalyzes preferably the condensation reaction of desaturated isoprenoid-diphosphates with homogentisic acid irregardless of the chain length. Carotenoids are also upregulated.

TABLE 1 – Vitamin E content in BY-2 lines transformed with pC1390PQ-35S (T), compared to the not transformed wild type (wt)

Sample	Dry weight (mg)	α-tocotrienol (ng/mg dry weight)	δ-tocopherol (ng/mg dry weight)
wt 5/10	293	764.89	321.91
wt 12/10	306	716.64	308.36
wt 19/10	300	623.42	322.78
T1 5/10	308	2824.90	304.44
T2 12/10	303	3253.99	289.05
T3 19/10	302	3184.36	368.71
T2 5/10	299	2758.32	202.00
T2 12/10	303	2831.79	223.05
T2 19/10	298	3911.06	370.11
T3 5/10	300	2309.44	319.81
T3 12/10	306	2488.68	377.60
T3 19/10	300	2605.15	350.65

TABLE 2 - Relative Vitamin E content in wild-type and transformed (pC1390PQ-35S) BY-2 cells.

Sample BY-2	α-tocotrienol+/- standard deviation (ng/mg dry weight)	δ-tocopherol +/- standard deviation (ng/mg dry weight)	relation α-tocotrienol (%)	relation δ-tocopherol (%)
wt	1.30+/-0.13	0.32 +/- 0.01	100 +/- 10	100 +/- 3
T1	4.88 +/- 0.52	0.28 +/- 0.07	375 +/- 40	88 +/- 22
T2	5.30+/-0.71	0.30 +/- 0.08	408 +/- 55	94 +/- 25
T3	6.38 +/- 1.35	0.37 +/- 0.01	491 +/- 103	116 +/- 3

TABLE 3 – Plastoquinone-9 content in BY-2 lines transformed with pC1390PQ-35S

Sample	dry weight (mg)	plastoquinone-9 (pmoles/mg dry weight)
Wt 5/10	293	27.71
Wt 12/10	306	38.28
Wt 19/10	300	28.58
T1 5/10	308	45.41
T2 12/10	303	50.18
T3 19/10	302	57.88
T2 5/10	299	47.29
T2 12/10	303	43.60
T2 19/10	298	54.31
T3 5/10	300	45.24
T3 12/10	306	45.13
T3 19/10	300	47.33

TABLE 4 - Relative plastoquinone-9 content in wild-type and transformed (pC1390PQ-35S) BY-2 cells.

Sample BY-2	plastoquinone-9 +/- standard deviation (pmoles/mg dry weight)	relation plastoquinone-9 (%)
wt	31.52 +/- 5.87	100 +/- 19
T1	45.98 +/- 1.14	146 +/- 4
T2	46.31 +/- 3.45	147+/- 11
T3	53.17 +/- 5.37	169 +/- 17

TABLE 5 – Carotenoid content in BY-2 lines transformed with pC1390PQ-35S

Sample	dry weight (mg)	carotenoid (pmoles/mg dry weight)
wt 5/10	293	45.78
wt 12/10	306	51.81
wt 19/10	300	45.41
T1 5/10	308	109.61
T2 12/10	303	97.27
T3 19/10	302	105.63
T2 5/10	299	112.69
T2 12/10	303	130.78
T2 19/10	298	103.48
T3 5/10	300	85.39
T3 12/10	306	87.55
T3 19/10	300	89.81

TABLE 6 - Relative carotenoid content in wild-type and transformed (pCPQ1390-35S) BY-2 cells.

Sample BY-2	carotenoid +/- standard deviation (pmoles/mg dry weight)	relation carotenoid (%)
wt	47.67 +/- 3.59	100 +/- 8
T1	128.33 +/- 14.96	215 +/- 31
T2	105.20 +/- 22.68	221 +/- 48
T3	99.64 +/- 8.58	209 +/- 18

Example 3

Preparation of transgenic *A. thaliana* plants

Wild-type *A.thaliana* plants (Columbia) are transformed with the *Agrobacterium tumefaciens* strain (EHA105), transformed with vector pC1390PQ-35S, on the basis of a modified method (Steve Clough and Andrew Bent. Floral dip: a simplified method for Agrobacterium mediated transformation of *A.thaliana*. Plant J 16(6):735-43, 1998) of the vacuum infiltration method according to Bechtold and colleagues (Bechtold, N. Ellis, J. and Pelltier, G.,in planta Agrobacteri-

um-mediated gene transfer by infiltration of adult *A.thaliana* plants. CRAcad Sci Paris, 1993. 1144(2):204-212).

Seeds of the primary transformants are selected on the basis of their resistance to antibiotics. Antibiotics-resistant seedlings were planted into soil and used for biochemical analysis as fully developed plants.

Example 4

Preparation of transgenic *Brassica napus* plants

Transgenic oilseed rape plants are prepared following a protocol by Bade, J.B. and Damm,B. (in Gene Transfer to Plants, Potrykus, I. and Spangenberg, G., eds, Springer Lab Manual, Springer Verlag, 1995, 30-38), which also indicates the composition of the media and buffers used. Transformations are carried out using the *Agrobacterium tumefaciens* strains EHA105 and GV3101, transformed with vector pC1390PQ-35S.

Seeds of *Brassica napus* var. Westar are surface-sterilized with 70% ethanol (v/v), washed in water at 55_C for 10 minutes, incubated in 1% strength hypochlorite solution (25% v/v Teepol, 0.1% v/v Tween 20) for 20 minutes and washed with sterile water six times for 20 minutes each. The seeds are dried for three days on filter paper, and 10-15 seeds were germinated in a glass flask containing 15 ml of germination medium. The roots and apices are removed from several seedlings (approx. size 10 cm) and the remaining hypocotyls are cut into pieces of approx. 6 mm in length. The approx. 600 explants obtained in this way are washed in 50 ml of basal medium for 30 minutes and then transferred into a 300 ml flask. After adding 100 ml of callus induction medium, the cultures are incubated at 100 rpm for 24 hours.

An overnight culture of the Agrobacterium strain is set up in Luria broth medium supplemented with canamycin (20 mg/l) at 29_C, and 2 ml of this are incubated in 50 ml of Luria broth medium without canamycin at 29_C for 4 hours until an OD_{600} of 0.4-0.5 was reached. After pelleting the culture at 2000 rpm for 25 min, the cell pellet is resuspended in 25 ml of basal medium. The concentration of the bacteria in the solution was adjusted to OD_{600} 0.3 by adding more basal medium.

The callus induction medium is removed from the oilseed rape explants using sterile pipettes, 50 ml of agrobacterial solution are added, and the reaction mixture is mixed carefully and incubated for 20 min. The agrobacterial suspension is removed, the oilseed rape explants are washed with 50 ml of callus induction medium for 1 min, and then 100 ml of callus induction medium are added. Cocultivation is carried out on an orbital shaker at 100 rpm for 24 h. Coculti-

vation is stopped by removing the callus induction medium and the explants are washed twice with 25 ml of washing medium for 1 min each and twice with 100 ml of washing medium for 60 min each at 100 rpm. The washing medium containing the explants is transferred into 15 cm Petri dishes and the medium is removed using sterile pipettes.

For regeneration, in each case 20-30 explants are transferred into 90 mm Petri dishes containing 25 ml of shoot induction medium with canamycin. The Petri dishes are sealed with 2 layers of Leukopor and incubated at 25_C and 2000 lux at photoperiods of 16 hours of light/8 hours of darkness. Every 12 days, the developing calli are transferred to fresh Petri dishes containing shoot induction medium. All further steps for the regeneration of whole plants are carried out as described by Bade, J.B and Damm, B. (in Gene Transfer to Plants, Potrykus, I. and Spangenberg, G., eds, Springer Lab Manual, Springer Verlag, 1995, 30-38).

Example 5

Preparation of transgenic *Nicotiana tabacum* plants

Ten ml of YEB medium with antibiotic (5 g/l beef extract, 1 g/l yeast extract, 5 g/l peptone, 5 g/l sucrose and 2 mM $MgSO_4$) are inoculated with an *Agrobacterium tumefaciens* colony, transformed with vector pC1390PQ-35S and cultured at 28 °C. The cells are pelleted in a benchtop centrifuge at 4 °C, 3500 rpm for 20 min and then resuspended in fresh YEB medium without antibiotics under sterile conditions. The cell suspension is used for transformation.

The sterile-cultured wild-type plants are obtained by vegetative replication. For this purpose, only the tip of the plant is cut off and transferred to fresh 2MS medium in a sterile preserving jar. The hairs on the upper side of the leaves and the central veins of the leaves are removed from the rest of the plant. Using a razor blade the leaves are cut into approximately 1 cm^2 square pieces. The agrobacterial culture is transferred into a small Petri dish (2 cm diameter). The leaf pieces are briefly drawn through this solution and placed bottom down on 2MS medium in Petri dishes (9 cm diameter) so as to touch the medium. After two days in the dark at 25_C, the explants are transferred to plates containing callus induction medium and warmed to 28_C in a controlled-environment cabinet. The medium had to be changed every 7-10 days. As soon as calli formed, the explants are transferred onto shoot induction medium with Claforan (0.6% BiTec agar (g/v), 2.0 mg/l zeatin ribose, 0.02 mg/l naphthylacetic acid, 0.02 mg/l gibberelic acid, 0.25 g/ml Claforan, 1.6% glucose (g/v) and 50 mg/l canamycin) in sterile preserving jars. After about one month, organogenesis started and it is possible to cut off the shoots formed. The shoots were cultured on 2MS medium with Claforan and selection marker. As soon as a strong root ball has developed, it is possible to put the plants into pots containing seed compost.

Example 6

Characterization of transgenic plants

The tocopherol and tocotrienol contents in leaves and seeds of the plants of examples 3, 4 and 5, which have been transformed with the constructs described *(Arabidopsis thaliana, Brassica napus* and *Nicotiana tabacum*), are analyzed. For this purpose, the transgenic plants are cultured in a greenhouse and plants expressing the gene coding for *Arabidopsis thaliana* prenyltransferase are identified at the Northern level. The tocopherol content and the tocotrienol content in the leaves and seeds of these plants are determined.

For this purpose, the leaf material of plants is deep-frozen in liquid nitrogen immediately after sampling. The subsequent disruption of the cells is carried out by means of a stirring apparatus by three incubations of 15 minutes each in 100% methanol at 30_C and 1000 rpm in an Eppendorf shaker, and the supernatants obtained in each case were combined.

Further incubation steps reveal no further release of tocopherols or tocotrienols.

In order to avoid oxidation, the extracts obtained are analyzed directly after extraction with the aid of an HPLC system (Waters Allience 2690). Tocopherols and tocotrienols are separated via a reverse phase column (ProntoSil 200-3-C30$^{(R)}$, Bischoff) using a mobile phase of 100% methanol and identified on the basis of standards (Merck). The detection system is the fluorescence of the substances (excitation 295 nm, emission 320 nm) which is detected with the aid of a Jasco fluorescence detector FP 920.

We claim:

1. A protein with prenyltransferase activity comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 50 % identity at amino acid level with sequence SEQ. ID. NO. 2.

2. A nucleic acid encoding a protein as claimed in claim 1.

3. A nucleic acid as claimed in claim 2 encoding a protein from plants, cyanobacteria, mosses or algae.

4. A nucleic acid as claimed in claim 3, comprising the sequence shown in SEQ. ID. NO. 1.

5. A nucleic acid construct comprising a nucleic acid as claimed in any of claims 2 to 4 functionally linked to one or more regulatory signals which ensure transcription and translation in organisms.

6. A nucleic acid construct as claimed in claim 5, wherein the regulatory signals comprise one or more promoters which ensure transcription and translation in organisms.

7. A nucleic acid construct as claimed in either of claims 5 and 6, wherein regulatory signals ensuring transcription and translation in plants are used.

8. A nucleic acid construct as claimed in claim 7, additionally comprising a nucleic acid encoding a plastid transit peptide.

9. A method for producing vitamin E, plastoquinone and/or carotenoids by culturing organisms which have, compared to the wild type, an increased prenyltransferase activity, said prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

10. A method as claimed in claim 9, wherein the prenyltransferase activity is increased by increasing, compared to the wild type, the gene expression of a nucleic acid encoding a prenyltransferase, said prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

11. A method as claimed in claim 10, wherein gene expression is increased by introducing into the organism at least one nucleic acid encoding a prenyltransferase, said prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

12. A method as claimed in claim 11, wherein nucleic acids encoding prenyltransferases from plants are used.

13. A method as claimed in claim 12, wherein nucleic acids comprising the sequence SEQ. ID. NO. 1 are introduced.

14. A method as claimed in any of claims 9 to 13, wherein the organism used are plants, cyanobacteria, mosses or algae.

15. A method as claimed in any of claims 9 to 14, wherein, after culturing, the organism is harvested and then vitamin E, plastoquinone and/or carotenoids are isolated from said organism.

16. The use of proteins comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50 % identical at the amino acid level with the sequence SEQ. ID. NO. 2 and having the enzymic activity of a prenyltransferase as prenyltransferase.

17. The use of nucleic acids encoding proteins as claimed in claim 16 for expressing proteins which have a prenyltransferase activity.

18. A genetically modified organism, wherein the genetic modification increases, compared to a wild type, the activity of a prenyltransferase and said prenyltransferase comprises the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50 % identical at the amino acid level to the sequence SEQ. ID. NO. 2.

19. A genetically modified organism as claimed in claim 18, wherein the increase in prenyltransferase activity is caused by an increase, compared to the wild type, in gene expression of a nucleic acid encoding a prenyltransferase and said prenyltransferase comprises the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2.

20. A genetically modified organism as claimed in claim 19, wherein gene expression is increased by introducing into said organism at least one nucleic acid which encodes a protein comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50% identical at the amino acid level to the sequence SEQ. ID. NO. 2 and having the enzymatic property of a prenyltransferase.

21. A genetically modified organism, comprising at least one transgenic nucleic acid which encodes a prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50 % identical at the amino acid level to the sequence SEQ. ID. NO. 2.

22. A genetically modified organism, comprising at least two endogenous nucleic acids which encode a prenyltransferase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which is at least 50 % identical at the amino acid level to the sequence SEQ. ID. NO. 2.

23. A genetically modified organism as claimed in any of claims 18 to 22, which has an increased vitamin E, plastoquinone and/or carotenoid content compared to the wild type.

24. A genetically modified organism as claimed in any of claims 18 to 23, wherein the organism used are plants, cyanobacteria, mosses or algae.

25. A genetically modified plant as claimed in claim 24, wherein the plants are selected from the group marigold, sunflower, Arabidopsis, tobacco, red pepper, soybean, tomato, egg-plant, paprika, carrot, potato, corn, lettuce and cabbage species, cereals, alfalfa, oats, barley, rye, wheat, triticale, millet, rice, lucerne, flax, cotton, hemp, rape, canola, sugar beet, sugar cane, nut and vine species or wood plants.

26. Seeds of the genetically modified plants as claimed in claim 25.

27. The use of the genetically modified organisms as claimed in any of claims 18 to 25 as feed- and foodstuffs, for producing processed food, for producing vitamin E-containing extracts of said organisms or for preparing feed and food supplements.

28. A method for preparing genetically modified organisms as claimed in any of claims 18 to 25, wherein nucleic acids as claimed in any claims of claim 2 to 4 or nucleic acid constructs as

claimed in any of claims 5 to 8 are introduced into the genome of the starting organism.

29. A method for identifying herbicidal agents comprising

i. incubating, with at least one candidate compound, a plant prenyltransferase polypeptide under conditions allowing the binding of the candidate compound to the plant prenyltransferase; and

ii. selecting, by step ii), at least a candidate compound which binds to the plant prenyltransferase of step i); or

iii. selecting, by step iii), at least a candidate compound which reduces or blocks the activity of the plant prenyltransferase of step i); or

iv. selecting, by step iv), at least a candidate compound which inhibits or decreases transcription, translation or expression of the plant prenyltransferase of step i),

wherein the plant prenyltransferase comprises the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 50 % identity at amino acid level with sequence SEQ. ID. NO. 2..

30. A method as claimed in claim 29, wherein the plant prenyltransferase is encoded by a nucleic acid sequence comprising

a) a nucleic acid sequence shown in SEQ ID NO:1; or

b) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from the amino acid sequence shown in SEQ ID NO: 2 by back translation; or

c) a nucleic acid sequence which, owing to the degeneracy of the genetic code, can be deduced from a functional equivalent of the amino acid sequence shown in SEQ ID NO: 2, which has an identity with SEQ ID NO:2 of at least 50%, by back translation.

31. A method as claimed in claim 29 or 30 which comprises testing a candidate compound in a plant prenyltransferase inhibition assay.

32. A method as claimed in claim 31 which comprises

a) incubating, with a candidate compound, a plant prenyltransferase in a cell free system;

b) selecting, by step b), a candidate compound which decreases the activity of the plant prenyltransferase.

33. A method as claimed in claim 32, wherein the enzymatic activity of the plant prenyltransferase is determined in comparison to the activity of a plant prenyltransferase not incubated with the candidate compound.

34. A method as claimed in any of claims 29 to 33, wherein the substances are identified in a high-throughput screening.

35. An herbicidal agent identified via one of the methods as claimed in any of claims 29 to 34.

36. The use of a plant prenyltransferase as target for the identification of herbicidal agents, wherein the plant prenyltransferase comprises the amino acid sequence SEQ. ID. NO. 2 or a sequence derived from this sequence by substitution, insertion or deletion of amino acids which has at least 50 % identity at amino acid level with sequence SEQ. ID. NO. 2.

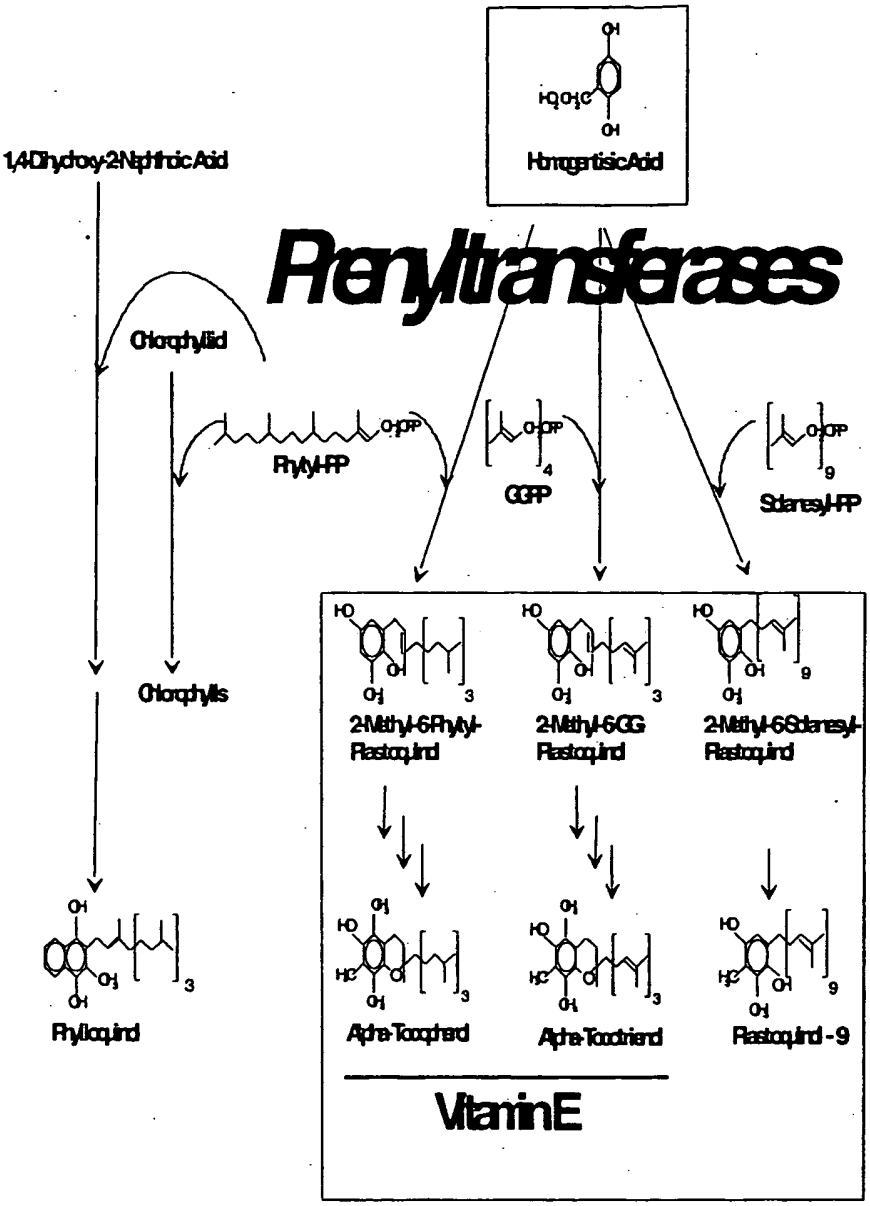

1,4-Dihydroxy-2-Naphthoic Acid
Homogentisic Acid
Prenyltransferases
Chlorophyllid
Phytyl-PP
GGPP
Solanesyl-PP
Chlorophylls
2-Methyl-6-Phytyl-Plastoquinol
2-Methyl-6-GG Plastoquinol
2-Methyl-6-Solanesyl-Plastoquinol
Phylloquinol
Alpha-Tocopherol
Alpha-Tocotrienol
Plastoquinol-9
Vitamin E

Figure 1

Figure 2

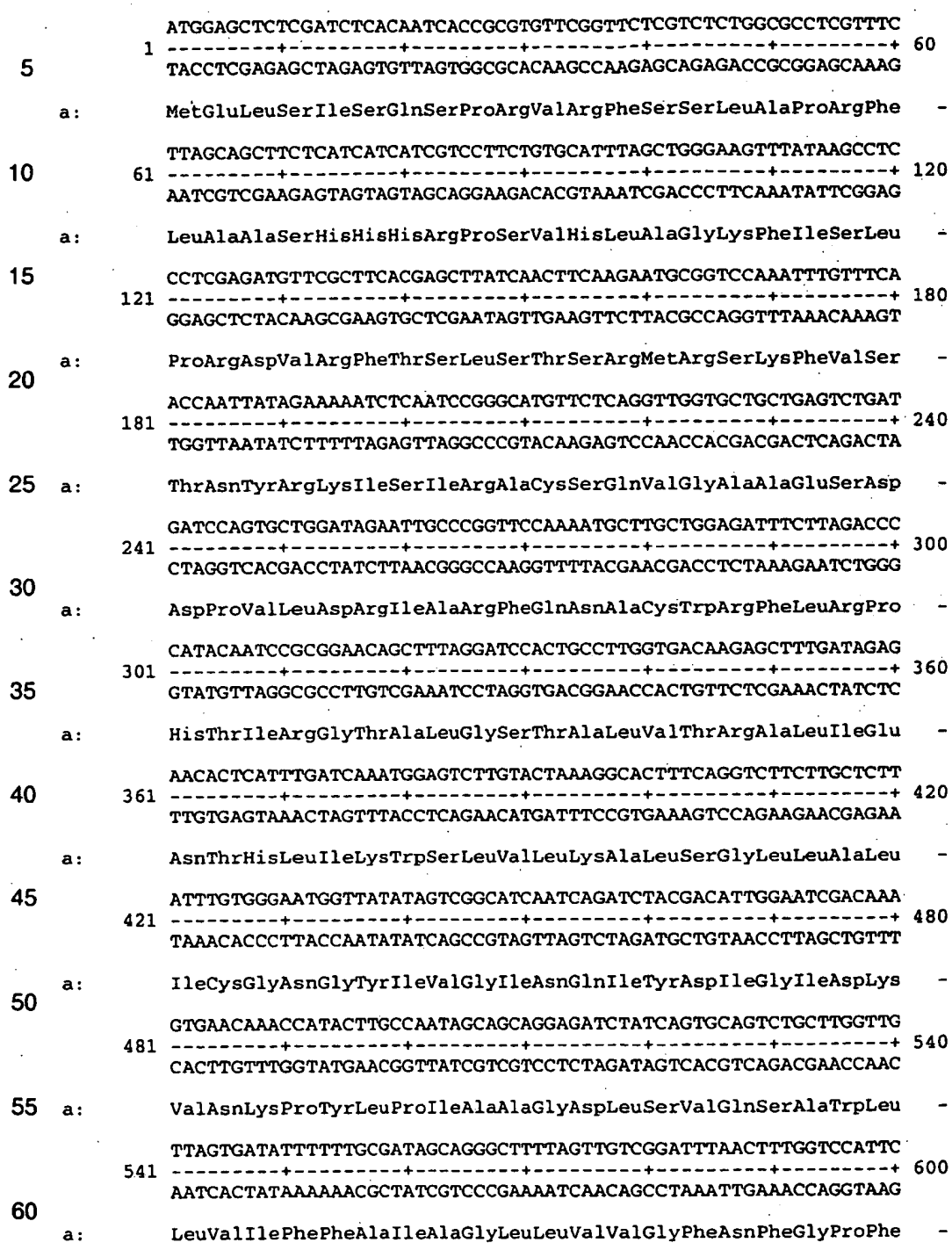

ATGGAGCTCTCGATCTCACAATCACCGCGTGTTCGGTTCTCGTCTCTGGCGCCTCGTTTC
1
60
TACCTCGAGAGCTAGAGTGTTAGTGGCGCACAAGCCAAGAGCAGAGACCGCGGAGCAAAG
a: MetGluLeuSerIleSerGlnSerProArgValArgPheSerSerLeuAlaProArgPhe -
TTAGCAGCTTCTCATCATCATCGTCCTTCTGTGCATTTAGCTGGGAAGTTTATAAGCCTC
61
120
AATCGTCGAAGAGTAGTAGTAGCAGGAAGACACGTAAATCGACCCTTCAAATATTCGGAG
a: LeuAlaAlaSerHisHisHisArgProSerValHisLeuAlaGlyLysPheIleSerLeu -
CCTCGAGATGTTCGCTTCACGAGCTTATCAACTTCAAGAATGCGGTCCAAATTTGTTTCA
121
180
GGAGCTCTACAAGCGAAGTGCTCGAATAGTTGAAGTTCTTACGCCAGGTTTAAACAAAGT
a: ProArgAspValArgPheThrSerLeuSerThrSerArgMetArgSerLysPheValSer -
ACCAATTATAGAAAAATCTCAATCCGGGCATGTTCTCAGGTTGGTGCTGCTGAGTCTGAT
181
240
TGGTTAATATCTTTTTAGAGTTAGGCCCGTACAAGAGTCCAACCACGACGACTCAGACTA
a: ThrAsnTyrArgLysIleSerIleArgAlaCysSerGlnValGlyAlaAlaGluSerAsp -
GATCCAGTGCTGGATAGAATTGCCCGGTTCCAAAATGCTTGCTGGAGATTTCTTAGACCC
241
300
CTAGGTCACGACCTATCTTAACGGGCCAAGGTTTTACGAACGACCTCTAAAGAATCTGGG
a: AspProValLeuAspArgIleAlaArgPheGlnAsnAlaCysTrpArgPheLeuArgPro -
CATACAATCCGCGGAACAGCTTTAGGATCCACTGCCTTGGTGACAAGAGCTTTGATAGAG
301
360
GTATGTTAGGCGCCTTGTCGAAATCCTAGGTGACGGAACCACTGTTCTCGAAACTATCTC
a: HisThrIleArgGlyThrAlaLeuGlySerThrAlaLeuValThrArgAlaLeuIleGlu -
AACACTCATTTGATCAAATGGAGTCTTGTACTAAAGGCACTTTCAGGTCTTCTTGCTCTT
361
420
TTGTGAGTAAACTAGTTTACCTCAGAACATGATTTCCGTGAAAGTCCAGAAGAACGAGAA
a: AsnThrHisLeuIleLysTrpSerLeuValLeuLysAlaLeuSerGlyLeuLeuAlaLeu -
ATTTGTGGGAATGGTTATATAGTCGGCATCAATCAGATCTACGACATTGGAATCGACAAA
421
480
TAAACACCCTTACCAATATATCAGCCGTAGTTAGTCTAGATGCTGTAACCTTAGCTGTTT
a: IleCysGlyAsnGlyTyrIleValGlyIleAsnGlnIleTyrAspIleGlyIleAspLys -
GTGAACAAACCATACTTGCCAATAGCAGCAGGAGATCTATCAGTGCAGTCTGCTTGGTTG
481
540
CACTTGTTTGGTATGAACGGTTATCGTCGTCCTCTAGATAGTCACGTCAGACGAACCAAC
a: ValAsnLysProTyrLeuProIleAlaAlaGlyAspLeuSerValGlnSerAlaTrpLeu -
TTAGTGATATTTTTTGCGATAGCAGGGCTTTTAGTTGTCGGATTTAACTTTGGTCCATTC
541
600
AATCACTATAAAAAACGCTATCGTCCCGAAAATCAACAGCCTAAATTGAAACCAGGTAAG
a: LeuValIlePhePheAlaIleAlaGlyLeuLeuValValGlyPheAsnPheGlyProPhe -

Figure 2 cont.

```
                ATTACAAGCCTATACTCTCTTGGCCTTTTTCTGGGAACCATCTATTCTGTTCCACCCCTC
      601 ---------+---------+---------+---------+---------+---------+ 660
                TAATGTTCGGATATGAGAGAACCGGAAAAAGACCCTTGGTAGATAAGACAAGGTGGGGAG

a:              IleThrSerLeuTyrSerLeuGlyLeuPheLeuGlyThrIleTyrSerValProProLeu   -

                AGAATGAAAAGATTCCCAGTTGCAGCATTTCTTATTATTGCCACGGTACGAGGTTTCCTT
      661 ---------+---------+---------+---------+---------+---------+ 720
                TCTTACTTTTCTAAGGGTCAACGTCGTAAAGAATAATAACGGTGCCATGCTCCAAAGGAA

a:              ArgMetLysArgPheProValAlaAlaPheLeuIleIleAlaThrValArgGlyPheLeu   -

                CTTAACTTTGGTGTGTACCATGCTACAAGAGCTGCTCTTGGACTTCCATTTCAGTGGAGT
      721 ---------+---------+---------+---------+---------+---------+ 780
                GAATTGAAACCACACATGGTACGATGTTCTCGACGAGAACCTGAAGGTAAAGTCACCTCA

a:              LeuAsnPheGlyValTyrHisAlaThrArgAlaAlaLeuGlyLeuProPheGlnTrpSer   -

                GCACCTGTGGCGTTCATCACATCTTTTGTGACACTGTTTGCACTGGTCATTGCTATTACA
      781 ---------+---------+---------+---------+---------+---------+ 840
                CGTGGACACCGCAAGTAGTGTAGAAAACACTGTGACAAACGTGACCAGTAACGATAATGT

a:              AlaProValAlaPheIleThrSerPheValThrLeuPheAlaLeuValIleAlaIleThr   -

                AAGGACCTTCCTGATGTTGAAGGAGATCGAAAGTTCCAAATATCAACCCTGGCAACAAAA
      841 ---------+---------+---------+---------+---------+---------+ 900
                TTCCTGGAAGGACTACAACTTCCTCTAGCTTTCAAGGTTTATAGTTGGGACCGTTGTTTT

a:              LysAspLeuProAspValGluGlyAspArgLysPheGlnIleSerThrLeuAlaThrLys   -

                CTTGGAGTGAGAAACATTGCATTCCTCGGTTCTGGACTTCTGCTAGTAAATTATGTTTCA
      901 ---------+---------+---------+---------+---------+---------+ 960
                GAACCTCACTCTTTGTAACGTAAGGAGCCAAGACCTGAAGACGATCATTTAATACAAAGT

a:              LeuGlyValArgAsnIleAlaPheLeuGlySerGlyLeuLeuLeuValAsnTyrValSer   -

                GCCATATCACTAGCTTTCTACATGCCTCAGGTTTTTAGAGGTAGCTTGATGATTCCTGCA
      961 ---------+---------+---------+---------+---------+---------+ 1020
                CGGTATAGTGATCGAAAGATGTACGGAGTCCAAAAATCTCCATCGAACTACTAAGGACGT

a:              AlaIleSerLeuAlaPheTyrMetProGlnValPheArgGlySerLeuMetIleProAla   -

                CATGTGATCTTGGCTTCAGGCTTAATTTTCCAGACATGGGTACTAGAAAAAGCAAACTAC
     1021 ---------+---------+---------+---------+---------+---------+ 1080
                GTACACTAGAACCGAAGTCCGAATTAAAAGGTCTGTACCCATGATCTTTTTCGTTTGATG

a:              HisValIleLeuAlaSerGlyLeuIlePheGlnThrTrpValLeuGluLysAlaAsnTyr   -

                ACCAAGGAAGCTATCTCAGGATATTATCGGTTTATATGGAATCTCTTCTACGCAGAGTAT
     1081 ---------+---------+---------+---------+---------+---------+ 1140
                TGGTTCCTTCGATAGAGTCCTATAATAGCCAAATATACCTTAGAGAAGATGCGTCTCATA

a:              ThrLysGluAlaIleSerGlyTyrTyrArgPheIleTrpAsnLeuPheTyrAlaGluTyr   -

                CTGTTATTCCCCTTCCTCTAG
     1141 ---------+---------+- 1161
                GACAATAAGGGGAAGGAGATC

a:              LeuLeuPheProPheLeuEnd   -
```

Figure 3

```
  1  MELSISQSPR VRFSSLAPRF LAASHHHRPS VHLAGKFISL PRDVRFTSLS
 51  TSRMRSKFVS TNYRKISIRA CSQVGAAESD DPVLDRIARF QNACWRFLRP
101  HTIRGTALGS TALVTRALIE NTHLIKWSLV LKALSGLLAL ICGNGYIVGI
151  NQIYDIGIDK VNKPYLPIAA GDLSVQSAWL LVIFFAIAGL LVVGFNFGPF
201  ITSLYSLGLF LGTIYSVPPL RMKRFPVAAF LIIATVRGFL LNFGVYHATR
251  AALGLPFQWS APVAFITSFV TLFALVIAIT KDLPDVEGDR KFQISTLATK
301  LGVRNIAFLG SGLLLVNYVS AISLAFYMPQ VFRGSLMIPA HVILASGLIF
351  QTWVLEKANY TKEAISGYYR FIWNLFYAEY LLFPFL*
```

Figure 4

```
Symbol comparison table: /gcg/gcg/gcgcore/data/rundata/blosum62.cmp
CompCheck: 1102

        Gap Weight:      8      Average Match:  2.778
     Length Weight:      2   Average Mismatch: -2.248

           Quality:   1737             Length:    441
             Ratio:  4.488               Gaps:      4
Percent Similarity: 95.866   Percent Identity: 94.574

      Match display thresholds for the alignment(s):
                 | = IDENTITY
                 : =   2
                 . =   1


        1 MELSISQSPRVRFSSLAPRFLAASHHHRPSVHLAGKFISLPRDVRFTSLS 50
          ||||||||||||||||||||||||||||||||||||||||||||||||||
        1 MELSISQSPRVRFSSLAPRFLAASHHHRPSVHLAGKFISLPRDVRFTSLS 50

       51 TSRMRSKFVSTNYRKISIR.................ACSQVGAAESDDPVL 84
          ||||||||||||||||||                 ||||||||||||||
       51 TSRMRSKFVSTNYRKISIRSVCAFCNGTHKSRYYQACSQVGAAESDDPVL 100

       85 DRIARFQNACWRFLRPHTIRGTALGSTALVTRALIENTHLIKWSLVLKAL 134
          ||||||||||||||||||||||||||||||||||||||||||||||||||
      101 DRIARFQNACWRFLRPHTIRGTALGSTALVTRALIENTHLIKWSLVLKAL 150

      135 SGLLALICGNGYIVGINQIYDIGIDKVNKPYLPIAAGDLSVQSAWLLVIF 184
          ||||||||||||||||||||||||||||||||||||||||||||||||||
      151 SGLLALICGNGYIVGINQIYDIGIDKVNKPYLPIAAGDLSVQSAWLLVIF 200

      185 FAIAGLLVVGFNFGPFITSLYSLGLFLGTIYSVPPLRMKRFPVAAFLIIA 234
          ||||||||||||||||||||||||||||||||||||||||||||||||||
      201 FAIAGLLVVGFNFGPFITSLYSLGLFLGTIYSVPPLRMKRFPVAAFLIIA 250

      235 TVRGFLLNFGVYHATRAALGLPFQWSAPVAFITSFVTLFALVIAITKDLP 284
          ||||||||||||||||||||||||||||||||||||||||||||||||||
      251 TVRGFLLNFGVYHATRAALGLPFQWSAPVAFITSFVTLFALVIAITKDLP 300

      285 DVEGDRKFQISTLATKLGVRNIAFLGSGLLLVNYVSAISLAFYMP..... 329
          |||||||||||||||||||||||||||||||||||||||||||
      301 DVEGDRKFQISTLATKLGVRNIAFLGSGLLLVNYVSAISLAFYMPQYAAL 350

      330 ...........QVFRGSLMIPAHVILASGLIFQTWVLEKANYTKE...AI 365
                     ||||||||||||||||||||||||||||||||
      351 KRPTLLSFNNEQVFRGSLMIPAHVILASGLIFQTWVLEKANYTKSICYSP 400

      366 SGYYRFIW.NLFYAEYLLFPFL*.................. 387
          |  :.|   .: :. |::   |
      401 SSSFQFHGEDMQFSLYIILLLLCSLESKSVPSCTYIKDMSF 441
```

Figure 5

```
Symbol comparison table: /gcg/gcg/gcgcore/data/rundata/blosum62.cmp
CompCheck: 1102

        Gap Weight:      8     Average Match:  2.778
     Length Weight:      2  Average Mismatch: -2.248

           Quality:    494            Length:    400
             Ratio:  1.604              Gaps:      8
Percent Similarity: 48.814  Percent Identity: 38.305

      Match display thresholds for the alignment(s):
                 | = IDENTITY
                 : =   2
                 . =   1

 51 TSRMRSKFVSTNYRKISIRACSQVGAAESDDPVLDRIARFQNACWRFLRP 100
                                          .|  | | ||| ||
  1 ..................................MATIQ.AFWRFSRP  13

101 HTIRGTALGSTALVTRALIENTHLIKWSLVLKALSGL.LALICGNGYIVG 149
    ||| || |   |.    :: . . :     |  . |  || : || ||||
 14 HTIIGTTLSVWAVYLLTILGDGNSVNSPASLDLVFGAWLACLLGNVYIVG  63

150 INQIYDIGIDKVNKPYLPIAAGDLSV.QSAWLLVIFFAIAGLLVVGFNFG 198
    :||::|: ||::||| ||:| || |: |  | :|    :|  | : .  |
 64 LNQLWDVDIDRINKPNLPLANGDFSIAQGRW.IVGLCGVAS.LAIAWGLG 111

199 PFITSLYSLGLFLGTIYSVPPLRMKRFPVAAFLIIATVRGFLLNFGVYHA 248
    .:     : | :|| |||||.|:||| . | | | |||| ..| |.:
112 LWLGLTVGISLIIGTAYSVPPVRLKRFSLLAALCILTVRGIVVNLGLFLF 161

249 TRAALGLPFQWSAPVAFITSFVTLFALVIAITKDLPDVEGDRKFQISTLA 298
     |  || |      |:  :| |: .| . ||| ||.||.||||.|.| ||
162 FRIGLGYPPTLITPIWVLTLFILVFTVAIAIFKDVPDMEGDRQFKIQTLT 211

299 TKLGVRNIAFLGSGLLLVN.YVSAISLAFYMPQVFRGSLMIPAHVILASG 347
    .:| .|: | |. :||   |..      :        . :| .|. | .
212 LQIGKQNV.FRGTLILLTGCYLAMAIWGLWAAMPLNTAFLIVSHLCLLAL 260

348 LIFQTWVLEKANYTKEAISGYYRFIWNLFYAEYLLFPFL*..........  387
    | ...  .   . |.  |. :|.||| ||: ||||:|
261 LWWRSRDVHLESKTE..IASFYQFIWKLFFLEYLLYPLALWLPNFSNTIF 308
```

Figure 6

```
Symbol comparison table: /gcg/gcg/gcgcore/data/rundata/blosum62.cmp
CompCheck: 1102

        Gap Weight:      8      Average Match:  2.778
     Length Weight:      2   Average Mismatch: -2.248

           Quality:    430             Length:    399
             Ratio:  1.111               Gaps:     12
Percent Similarity: 41.470   Percent Identity: 33.858

      Match display thresholds for the alignment(s):
                  | = IDENTITY
                  : =   2
                  . =   1

/lfs/people/un78pb/schledzco/PQPkorr_114.translate x
/lfs/people/un78pb/schledzco/HPT June 28, 2002 13:59  ..

      1 MELSISQSPRVRFS.SLAPRFLAASHHHRPSVHL....AGKFISLPRDVR 45
        ||  :| |  |  .    :      |    : .    . | :. |:  |
      1 MESLLSSSSLVSAAGGFCWKKQNLKLHSLSEIRVLRCDSSKVVAKPK.FR 49

     46 FTSLSTSRMRSKFVSTNYRKISIRACSQVGAAES.DDPVLDRIARFQ.NA 93
        .      |  .     |   |  .  |  |. |     :  |   .|
     50 NNLVRPDGQGSSLLLYPKHKSRFRVNATAGQPEAFDSNSKQKSFRDSLDA 99

     94 CWRFLRPHTIRGTALGSTALVTRALIENTHLIKWSLVLKALSGLLALICG 143
         :|| |||| : || | |   |.    |   |   |    | ..| :
    100 FYRFSRPHTVIGTVL.SILSVSFLAAEKVSDISPLLFTGILEAVVAALMM 148

    144 NGYIVGINQIYDIGIDKVNKPYLPIAAGDLSVQSAWLLVIFFAIAGLLVV 193
        | ||||:||: |: ||||||||||:|.|: || .   :|  |.|     .
    149 NIYIVGLNQLSDVEIDKVNKPYLPLASGEYSVNTGIAIVASFSIMSFW.L 197

    194 GFNFG..PFITSLYSLGLFLGTIYSV..PPLRMKRFPVAAFLIIATVRGF 239
        |.  |  |   .|: .   ||| ||:  | || ||| . | : |  ||
    198 GWIVGSWPLFWALF.VSFMLGTAYSINLPLLRWKRFALVAAMCILAVRAI 246

    240 LLNFGVY.HATRAALGLPFQWSAPVAFITSFVTLFALVIAITKDLPDVEG 288
        :.    | |       | |  .. |. | |.|.. |..|||: ||:||:||
    247 IVQIAFYLHIQTHVFGRPILFTRPLIFATAFMSFFSVVIALFKDIPDIEG 296

    289 DRKFQISTLATKLGVRNIAFLGSGLLLVNYVSAISLAFYMPQVFRGSLMI 338
        |: | | . .  || : : .    || . |  || .    | :.   : :
    297 DKIFGIRSFSVTLGQKRVFWTCVTLLQMAYAVAILVGATSPFIWSKVISV 346

    339 PAHVILASGLIFQTWVLEKANYTKEAISGYYRFIWNLFYAEYLLFPFL* 387
          |||||. |   .   .: .. |.  |.  | ||| |||||||| |||
    347 VGHVILATTLWARAKSVDLSSKTE..ITSCYMFIWKLFYAEYLLLPFLK 393
```

Figure 7

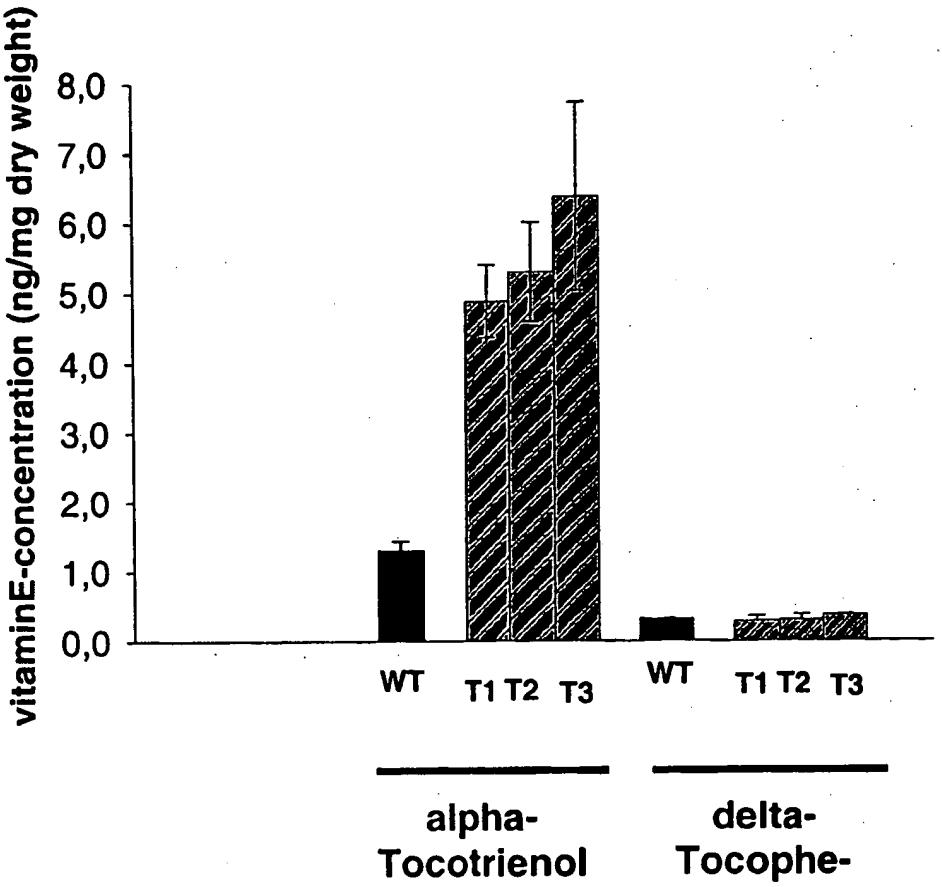
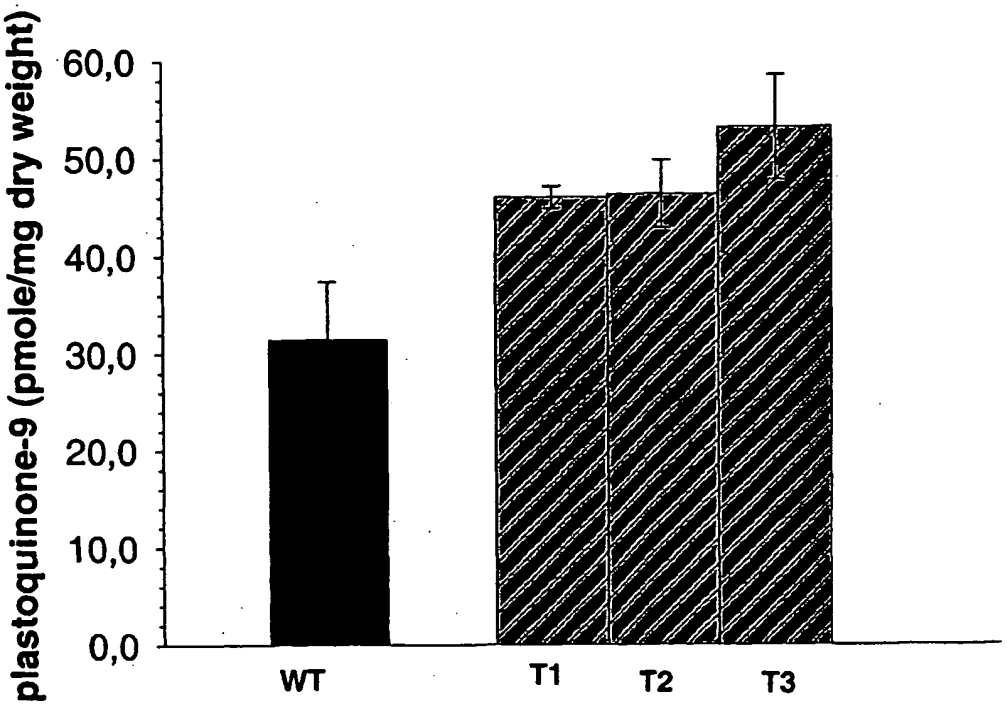

vitaminE-concentration (ng/mg dry weight)
8,0
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
WT
T1 T2 T3
WT
T1 T2 T3
alpha-
Tocotrienol
delta-
Tocophe-

Figure 8


plastoquinone-9 (pmole/mg dry weight)
60,0
50,0
40,0
30,0
20,0
10,0
0,0
WT
T1
T2
T3

Prenyltransferase.ST25.txt
SEQUENCE LISTING

<110> SunGene GmbH & Co. KGaA

<120> Novel Prenyltransferase

<130> AE 905/2002

<160> 15

<170> PatentIn version 3.1

<210> 1
<211> 1161
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)..(1158)
<223>

<220>
<221> misc_feature
<222> (75)..(91)
<223> motive 1

<220>
<221> misc_feature
<222> (464)..(478)
<223> motive 2

<400> 1
atg gag ctc tcg atc tca caa tca ccg cgt gtt cgg ttc tcg tct ctg 48
Met Glu Leu Ser Ile Ser Gln Ser Pro Arg Val Arg Phe Ser Ser Leu
1 5 10 15

```
gcg cct cgt ttc tta gca gct tct cat cat cat cgt cct tct gtg cat      96
Ala Pro Arg Phe Leu Ala Ala Ser His His His Arg Pro Ser Val His
            20                  25                  30

tta gct ggg aag ttt ata agc ctc cct cga gat gtt cgc ttc acg agc     144
Leu Ala Gly Lys Phe Ile Ser Leu Pro Arg Asp Val Arg Phe Thr Ser
        35                  40                  45

tta tca act tca aga atg cgg tcc aaa ttt gtt tca acc aat tat aga     192
Leu Ser Thr Ser Arg Met Arg Ser Lys Phe Val Ser Thr Asn Tyr Arg
    50                  55                  60

aaa atc tca atc cgg gca tgt tct cag gtt ggt gct gct gag tct gat     240
Lys Ile Ser Ile Arg Ala Cys Ser Gln Val Gly Ala Ala Glu Ser Asp
65                  70                  75                  80

gat cca gtg ctg gat aga att gcc cgg ttc caa aat gct tgc tgg aga     288
Asp Pro Val Leu Asp Arg Ile Ala Arg Phe Gln Asn Ala Cys Trp Arg
                85                  90                  95

ttt ctt aga ccc cat aca atc cgc gga aca gct tta gga tcc act gcc     336
Phe Leu Arg Pro His Thr Ile Arg Gly Thr Ala Leu Gly Ser Thr Ala
            100                 105                 110

ttg gtg aca aga gct ttg ata gag aac act cat ttg atc aaa tgg agt     384
Leu Val Thr Arg Ala Leu Ile Glu Asn Thr His Leu Ile Lys Trp Ser
        115                 120                 125

ctt gta cta aag gca ctt tca ggt ctt ctt gct ctt att tgt ggg aat     432
Leu Val Leu Lys Ala Leu Ser Gly Leu Leu Ala Leu Ile Cys Gly Asn
    130                 135                 140

ggt tat ata gtc ggc atc aat cag atc tac gac att gga atc gac aaa     480
Gly Tyr Ile Val Gly Ile Asn Gln Ile Tyr Asp Ile Gly Ile Asp Lys
145                 150                 155                 160

gtg aac aaa cca tac ttg cca ata gca gca gga gat cta tca gtg cag     528
Val Asn Lys Pro Tyr Leu Pro Ile Ala Ala Gly Asp Leu Ser Val Gln
                165                 170                 175

tct gct tgg ttg tta gtg ata ttt ttt gcg ata gca ggg ctt tta gtt     576
Ser Ala Trp Leu Leu Val Ile Phe Phe Ala Ile Ala Gly Leu Leu Val
            180                 185                 190

gtc gga ttt aac ttt ggt cca ttc att aca agc cta tac tct ctt ggc     624
Val Gly Phe Asn Phe Gly Pro Phe Ile Thr Ser Leu Tyr Ser Leu Gly
        195                 200                 205

ctt ttt ctg gga acc atc tat tct gtt cca ccc ctc aga atg aaa aga     672
Leu Phe Leu Gly Thr Ile Tyr Ser Val Pro Pro Leu Arg Met Lys Arg
    210                 215                 220

ttc cca gtt gca gca ttt ctt att att gcc acg gta cga ggt ttc ctt     720
Phe Pro Val Ala Ala Phe Leu Ile Ile Ala Thr Val Arg Gly Phe Leu
225                 230                 235                 240

ctt aac ttt ggt gtg tac cat gct aca aga gct gct ctt gga ctt cca     768
Leu Asn Phe Gly Val Tyr His Ala Thr Arg Ala Ala Leu Gly Leu Pro
                245                 250                 255

ttt cag tgg agt gca cct gtg gcg ttc atc aca tct ttt gtg aca ctg     816
Phe Gln Trp Ser Ala Pro Val Ala Phe Ile Thr Ser Phe Val Thr Leu
            260                 265                 270

ttt gca ctg gtc att gct att aca aag gac ctt cct gat gtt gaa gga     864
Phe Ala Leu Val Ile Ala Ile Thr Lys Asp Leu Pro Asp Val Glu Gly
        275                 280                 285
```

```
gat cga aag ttc caa ata tca acc ctg gca aca aaa ctt gga gtg aga      912
Asp Arg Lys Phe Gln Ile Ser Thr Leu Ala Thr Lys Leu Gly Val Arg
    290                 295                 300

aac att gca ttc ctc ggt tct gga ctt ctg cta gta aat tat gtt tca      960
Asn Ile Ala Phe Leu Gly Ser Gly Leu Leu Leu Val Asn Tyr Val Ser
305                 310                 315                 320

gcc ata tca cta gct ttc tac atg cct cag gtt ttt aga ggt agc ttg     1008
Ala Ile Ser Leu Ala Phe Tyr Met Pro Gln Val Phe Arg Gly Ser Leu
                325                 330                 335

atg att cct gca cat gtg atc ttg gct tca ggc tta att ttc cag aca     1056
Met Ile Pro Ala His Val Ile Leu Ala Ser Gly Leu Ile Phe Gln Thr
            340                 345                 350

tgg gta cta gaa aaa gca aac tac acc aag gaa gct atc tca gga tat     1104
Trp Val Leu Glu Lys Ala Asn Tyr Thr Lys Glu Ala Ile Ser Gly Tyr
        355                 360                 365

tat cgg ttt ata tgg aat ctc ttc tac gca gag tat ctg tta ttc ccc     1152
Tyr Arg Phe Ile Trp Asn Leu Phe Tyr Ala Glu Tyr Leu Leu Phe Pro
    370                 375                 380

ttc ctc tag                                                        1161
Phe Leu
385
```

<210> 2

<211> 386

<212> PRT

<213> Arabidopsis thaliana

<220>

<221> misc_feature

<222> (75)..(91)

<223> motive 1

<220>

<221> misc_feature

<222> (464)..(478)

<223> motive 2

<400> 2

```
Met Glu Leu Ser Ile Ser Gln Ser Pro Arg Val Arg Phe Ser Ser Leu
1               5                   10                  15

Ala Pro Arg Phe Leu Ala Ala Ser His His His Arg Pro Ser Val His
            20                  25                  30

Leu Ala Gly Lys Phe Ile Ser Leu Pro Arg Asp Val Arg Phe Thr Ser
        35                  40                  45
```

```
Leu Ser Thr Ser Arg Met Arg Ser Lys Phe Val Ser Thr Asn Tyr Arg
    50                  55                  60

Lys Ile Ser Ile Arg Ala Cys Ser Gln Val Gly Ala Ala Glu Ser Asp
65                  70                  75                  80

Asp Pro Val Leu Asp Arg Ile Ala Arg Phe Gln Asn Ala Cys Trp Arg
                85                  90                  95

Phe Leu Arg Pro His Thr Ile Arg Gly Thr Ala Leu Gly Ser Thr Ala
            100                 105                 110

Leu Val Thr Arg Ala Leu Ile Glu Asn Thr His Leu Ile Lys Trp Ser
        115                 120                 125

Leu Val Leu Lys Ala Leu Ser Gly Leu Leu Ala Leu Ile Cys Gly Asn
    130                 135                 140

Gly Tyr Ile Val Gly Ile Asn Gln Ile Tyr Asp Ile Gly Ile Asp Lys
145                 150                 155                 160

Val Asn Lys Pro Tyr Leu Pro Ile Ala Ala Gly Asp Leu Ser Val Gln
                165                 170                 175

Ser Ala Trp Leu Leu Val Ile Phe Phe Ala Ile Ala Gly Leu Leu Val
            180                 185                 190

Val Gly Phe Asn Phe Gly Pro Phe Ile Thr Ser Leu Tyr Ser Leu Gly
        195                 200                 205

Leu Phe Leu Gly Thr Ile Tyr Ser Val Pro Pro Leu Arg Met Lys Arg
    210                 215                 220

Phe Pro Val Ala Ala Phe Leu Ile Ile Ala Thr Val Arg Gly Phe Leu
225                 230                 235                 240

Leu Asn Phe Gly Val Tyr His Ala Thr Arg Ala Ala Leu Gly Leu Pro
                245                 250                 255

Phe Gln Trp Ser Ala Pro Val Ala Phe Ile Thr Ser Phe Val Thr Leu
            260                 265                 270

Phe Ala Leu Val Ile Ala Ile Thr Lys Asp Leu Pro Asp Val Glu Gly
        275                 280                 285

Asp Arg Lys Phe Gln Ile Ser Thr Leu Ala Thr Lys Leu Gly Val Arg
    290                 295                 300

Asn Ile Ala Phe Leu Gly Ser Gly Leu Leu Leu Val Asn Tyr Val Ser
305                 310                 315                 320
```

Ala Ile Ser Leu Ala Phe Tyr Met Pro Gln Val Phe Arg Gly Ser Leu
325 330 335

Met Ile Pro Ala His Val Ile Leu Ala Ser Gly Leu Ile Phe Gln Thr
340 345 350

Trp Val Leu Glu Lys Ala Asn Tyr Thr Lys Glu Ala Ile Ser Gly Tyr
355 360 365

Tyr Arg Phe Ile Trp Asn Leu Phe Tyr Ala Glu Tyr Leu Leu Phe Pro
370 375 380

Phe Leu
385

<210> 3

<211> 36

<212> DNA

<213> Artifical Sequence Primer PQ5

<220>

<221> primer_bind

<222> (1)..(36)

<223>

<400> 3
aagcttctgg agctgtcgat ctcacaatca ccgcgt 36

<210> 4

<211> 36

<212> DNA

<213> Artifical Sequence Primer PQ3

<220>

<221> primer_bind

<222> (1)..(36)

<223>

<400> 4
catatcgtcg acatgaaatt gaaagctaga ggaagg 36

<210> 5
<211> 36
<212> DNA
<213> Artifical Sequence Primer PQBY5

<220>
<221> primer_bind
<222> (1)..(36)
<223>

<400> 5
gcccttcccg ggatggagct gtcgatctca caatca 36

<210> 6
<211> 36
<212> DNA
<213> Artifical Sequence Primer PQBY3

<220>
<221> primer_bind
<222> (1)..(36)
<223>

<400> 6
tgaaatgaat tcctagagga aggggaataa cagata 36

<210> 7
<211> 1100
<212> DNA
<213> Brassica napus

<220>
<221> EST
<222> (1)..(1100)
<223>

<400> 7
atggagctct cgatctcaca atcaccgcgt gttcggttct cgtctctggc gcctcgtttc 60
ttagcagctt ctcatcatca tcgtccttct gtgcatttag ctgggaagtt tataagcctc 120
cctcgagatg ttcgcttcac gagcttatca acttcaagaa tgcggtccaa atttgtttca 180
accaattata gaaaaatctc aatccgggca tgttctcagg ttggtgctgc tgagtctgat 240
gatccagtgc tggatagaat tgcccggttc caaaatgctt gctggagatt tcttagaccc 300
catacaatcc gcggaacagc tttaggatcc actgccttgg tgacaagagc tttgatagag 360
aacactcatt tgatcaaatg gagtcttgta ctaaaggcac tttcaggtct tcttgctctt 420
atttgtggga atggttatat agtcggcatc aatcagatct acgacattgg aatcgacaaa 480
gtgaacaaac catacttgcc aatagcagca ggagatctat cagtgcagtc tgcttggttg 540
ttagtgatat tttttgcgat agcagggctt ttagttgtcg gatttaactt tggtccattc 600
attacaagcc tatactctct tggccttttt ctgggaacca tctattctgt tccacccctc 660
agaatgaaaa gattcccagt tgcagcattt cttattattg ccacggtacg aggtttcctt 720
cttaactttg gtgtgtacca tgctacaaga gctgctcttg gacttccatt tcagtggagt 780
gcacctgtgg cgttcatcac atcttttgtg acactgtttg cactggtcat tgctattaca 840
aaggaccttc ctgatgttga aggagatcga aagttccaaa tatcaaccct ggcaacaaaa 900
cttggagtga gaaacattgc attgcctcag gtttttagag gtagcttgat gattcctgca 960
catgtgatct tggcttcagg cttaattttc cagacatggg tactagaaaa agcaaactac 1020
accaaggaag ctatctcagg atattatcgg tttatatgga atctcttcta cgcagagtat 1080
ctgttattcc ccttcctcta 1100

<210> 8
<211> 323
<212> DNA
<213> Brassica napus

<220>
<221> misc_feature
<222> (1)..(323)
<223> "n" = unidentified

<400> 8
ataaacgtcc agnattccag gtcgacgatt tcgtgagtct agtactaaaa gcactttcag 60
gtcttcttgc tcttatatgt ggaaatggtt atatagttgg catcaatcag atctacgaca 120
ttggaattga caagtgaaca aaccatactt gcatacagca gaatttatgc tctcggctgt 180

gtatattttg accatctgtt cgacaccctc cctcttccct ccgtcgctcc tacccccctcc 240
cccgcctccc ccccccaccc tccacccccc ccctcaaatc ccctcgccgc tttccccccc 300
cccttgcccc ttttacaacc ccg 323

<210> 9
<211> 475
<212> DNA
<213> Brassica napus

<220>
<221> EST
<222> (1)..(475)
<223>

<400> 9
ttacccgcaa gacatttgga attcaaaaac accaatcctt tagtatgtac gtgaacaaag 60
tgattttgtc tccaagctac aaagaagaag aagagaggta tacaaagggg actacaaatg 120
ttcaccatga atgctagaag aaggggaata acagatactc tgcgtagaag agattccata 180
taaaccggta atatcctgct atagcttcct ttgtgtagtt tgctttttct agcacccatg 240
tctggaaaac caagcatgaa gccaagatca tatgtgcagg aatcatcaag ctacctctaa 300
aaacctgagg catgtagaaa gctagtgata tggcagaaat atagttcact agcagaagtc 360
cagaaccgag gaatgcaatg ttcctcactc caagctttgt tgctagtgtt gatatttgga 420
acttgcgatc tccttcaaca tcaggaagat cttttgtaat agcaatgact agtgc 475

<210> 10
<211> 433
<212> DNA
<213> Brassica napus

<220>
<221> EST
<222> (1)..(433)
<223>

<400> 10
tcctcttcat cacctcgttt cttagcagct tcttctcatc attaccggcc ttctgtgcat 60

ttagctggga agcttttaag ccggtctaaa gatgctgact taacgagctt atcaagttct 120
tgtatgcggt cgaagtttgt ttcaactaac tacagaaaaa tctcaatcag ggcaagttct 180
caagtaggtg ctgctggttc ggatccagtt cttgatagac ttgcgcgttt ccagaacgct 240
tgctggagat ttcttaggcc acataccatc cgcggaacag ctttaggatc cactgccttg 300
gtgacaagag ctttgataga gaacactcat ctgatcaaat ggagcctcgt actcaaggcg 360
ctttcaggtc ttcttgctct tatatgtggg aatgggtata tagttggcat caatcagatc 420
tacgacattg gca 433

<210> 11
<211> 452
<212> DNA
<213> Brassica napus

<220>
<221> EST
<222> (1)..(452)
<223>

<400> 11
cgcggtggaa ttttcgggta gacgatcccg tgaaatggtt atattgttgg catcaatcat 60
atctaccaca ttgttattga caaagtgaac aaaccatact tgccagtagc agcaggagat 120
ctctcagtgc agtctgcgtg gctgttagtg atctttttcg caattgcagg gctattagtt 180
gtcggattca actttggtcc gttcattaca agcctatact ctcttggcct ctttcttggg 240
acaatctatt ctgttccacc attaagaatg aaacgatttc cagttgcagc gtttctcatt 300
atcgccacgg tacgaggttt ccttcttaat tttggtgtat accatgctac aagagctgcc 360
cttggacttt cgtttcagtg gagtgcacct gtggctttca taacgtcttt tgtgacactg 420
tttgcactgg tcattgctat tacaaaagat cg 452

<210> 12
<211> 415
<212> DNA
<213> Brassica napus

<220>
<221> EST

<222> (1)..(415)

<223>

<400> 12
ggctagtggc atcaatggag ctctcgatct cacattcacc ttgtcttcgg ttctcctctt 60
catcacctcg gttcttagca gcttcttctc atcatcatca tcggccttcg ttgcatttag 120
ctgggaagct tttaagcagg cctaaagatg ttggcttcac gagcttatca agttcgtgtt 180
tgcggtcgaa atttgtttca actggttata gaaaaatctc gatccgggca tgttctcagg 240
ttggtgctgc tggttcggat ccagttcttg atagaattgc gcgtttccaa aatgcttgct 300
ggagatttct taggccacat accatccgtg gaacagcttt aggatccact gccttggtta 360
caagagcttt gatagagaac actcatctca ttaagtggag tctcgtactc aaggc 415

<210> 13
<211> 433
<212> DNA
<213> Brassica napus

<220>
<221> EST
<222> (1)..(433)
<223>

<400> 13
ggtcgacgat ttcgtttgtg tggctagtgt catcaatgga gctctcgatc tcgcaatcgc 60
cacgtcttcg attctcgtct gcatcgcctg gtttctcagc agcttctcat catcatcatc 120
atcgaccttc ggtgcattta gctgggaggc ttttaacccg tcataaagat gctgccttta 180
cgagcttatc aagttcgtgt atgcagtcga aacttgtctc aactaattat agaaaaatct 240
cgatccgggc atgttctcag gtcggtgcag ctggttctga tccaattctg gatagaatct 300
cccgtttcca aaatgcttgc tggagatttc ttaggccaca tacgatccgt ggaactgctt 360
taggatcaac tgccttggtg gcaagagcgt tgatagagaa cactcatctt atcaagtgga 420
gtctagtact aaa 433

<210> 14
<211> 383
<212> DNA
<213> Brassica napus

<220>
<221> EST
<222> (1)..(383)
<223>

<400> 14
ccaatagcag caggagatct ctcagtgcag tctgcgtggc tgttggtgat atttttcgca 60
gttgcagggc taacagttgt tggagtcaac tttggtccat tcattacatg cctatactct 120
ctaggtcttt ttctgccgac catctattct gtatcaccat tgaaaatgaa caaatgtcca 180
ggtgcagcgt atcttattat ggccacggaa caaagtttac ttgacaatta tgttactcac 240
ccatccctcg cggaccttaa ccaaagatac atactcctga ccttcaactc cgccgtagac 300
tcacgtcctc tccattaccc cctgtctccc accttcgtac tcctgctcca tttgctattg 360
tcgcattcga cctacatacc ccg 383

<210> 15
<211> 572
<212> DNA
<213> Soya

<220>
<221> EST
<222> (1)..(572)
<223>

<400> 15
gcatcaatca aatctatgac attagcattg acaaggtaaa caaaccttat ttacctatag 60
ctgctggaga tctttctgtc caatctgcat ggttcttggt tatatttttt gcagctgctg 120
gcctgtcaat tgtggggttg aactttgggc ccttcatttt ttctctttac acacttggcc 180
ttttcttggg taccatctat tctgttcctc catttagaat gaaacgcttt cctgttgcag 240
catttcttat aattgccacg gttcggggtt ttctccttaa ctttggtgtg tactatgcca 300
ctagagctgc ccttgggctt gcatttgaat ggagctctcc tgkggttttt atcacaacat 360
ttgtaacatt tttcgcactg gtaattgcta taacaaaaga tcttcctgat gttgaaggag 420
atcgcaagta tcagatatca acctttgcca caaaattagg agttcggaac attgctttcc 480
ttggttctgg aattttgttg gagaactata ttgtttctgt tttggcagca atttatatgc 540
ctcaggcttt caggcgttgg ttactcatac cg 572